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Prevalence of fungi and their antifungal and disinfectant resistance in hospital environments: insights into combating nosocomial mycoses

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Abstract

Background Fungal infections are increasingly recognized as a global health concern, contributing to considerable morbidity and mortality in hospital settings. This underscores the urgent need for infection prevention and control in healthcare facilities to protect vulnerable patients from the risk of acquiring invasive fungal diseases (IFDs). Given the critical role of transmission-based precautions in limiting the spread of filamentous fungi responsible for IFDs, this study was conducted to explore the potential role of the hospital environment in the dissemination of these infections.

Methods A total of 83 samples were collected from the air and surface of exhaust vents in the intensive care units (ICUs) of hospitals in Isfahan, Iran, to assess the presence and diversity of fungal species. Susceptibility testing against antifungal agents, including commonly used drugs and disinfectants, was performed on the identified fungal isolates. Furthermore, the antifungal resistance profiles of isolates from clinical IFD cases were compared with those of environmental isolates.

Results Fungi were detected in 45% of air samples and 100% of exhaust vent samples, with *Aspergillus* species being the most commonly identified genus. Mucorales were also found in 17% of exhaust vent samples. *Aspergillus* spp. and *Rhizopus* spp. showed the highest resistance to Amphotericin B, and a considerable proportion of these isolates exhibited simultaneous resistance to disinfectants. A similar antifungal resistance profile was noted between *A. flavus* and some *R. arrhizus* isolates from both environmental and clinical samples.

Conclusions The findings of this study indicate that the hospital environment, particularly exhaust vents, may act as a significant reservoir for causative agents of IFDs. This highlights the importance of environmental surveillance in preventing and controlling nosocomial fungal infections.

Keywords Invasive fungal diseases, *Aspergillus*, Mucorales, Air, Antifungal drugs, Disinfectants

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Introduction

The incidence of hospital-acquired infections (HAIs) caused by pathogenic or potentially pathogenic microorganisms remains a significant global problem. In recent years, there has been an alarming increase in the global burden of mycoses, with fungal infections emerging as a leading cause of morbidity and mortality in hospital settings [1]. Fungi are responsible for a wide range of diseases, ranging from localized non-invasive conditions to invasive and disseminated infections. Among hospitalized patients with compromised immunity, such as those who have undergone bone marrow or solid organ transplants or those receiving chemotherapy for cancer, invasive fungal diseases (IFDs) pose a significant and potentially life-threatening risk. Recent studies estimate that fungal infections cause the death of more than 1.5 million people worldwide each year [2]. *Candida* and *Aspergillus* are the most common fungal species causing serious infections, accounting for approximately 15% of HAIs [3]. *Aspergillus* species, particularly *Aspergillus fumigatus*, rank as the second most frequent cause of nosocomial invasive fungal infections, with mortality rates ranging from 30 to 58% [4]. Mucormycosis, caused by a group of molds known as Mucorales, is another rare but fatal fungal infection [5, 6].

The emergence of COVID-19 has compounded the challenge of nosocomial fungal infections, as secondary fungal infections have been reported in some COVID-19 patients [3, 5, 7, 8]. It has been reported that COVID-19 patients admitted to the intensive care units and placed on mechanical ventilation face a heightened risk of developing invasive pulmonary aspergillosis and mucormycosis [9]. This underscores the critical importance of addressing nosocomial fungal infections in the context of the ongoing COVID-19 pandemic.

It is crucial to acknowledge that the inhalation of *Aspergillus* and Mucorales spores, which are commonly found in soil, air, and surfaces, is the primary route of transmission for IFDs in susceptible individuals. These molds release spores that can easily become aerosolized, spreading throughout hospital environments [1, 2, 4]. Therefore, assessing their presence and diversity in healthcare settings, especially in wards housing vulnerable patients, is essential [6].

Moreover, with the limited effectiveness of antifungal prophylaxis and the constrained activity of current antifungal drugs, preventing these infections is paramount [10]. The emergence of antifungal resistance is a growing concern that affects both patient outcomes and treatment efficacy [11]. Increasing antifungal resistance rates highlight the often-overlooked threat posed by fungi in hospital environments. Consequently, implementing preventive measures to reduce airborne fungal concentrations, and thereby lowering the risk of fungal infections, is imperative. Within the One Health framework, environmental surveillance serves as a valuable tool in identifying potential sources of antimicrobial resistant infections within hospitals and evaluating the effectiveness of environmental disinfection and other infection control measures [12]. It is important to note that sublethal exposure of microorganisms to disinfectants, as well as the misuse or overuse of antimicrobial agents, can trigger stress responses in microorganisms, promoting antimicrobial resistance [13]. While several studies worldwide, including in Iran, have examined the presence of fungi in hospital environments [14–17], limited research has focused on the drug resistance of fungi in these settings [14–16]. Significant knowledge gaps remain regarding the resistance profiles of fungi to antifungal drugs and disinfectants in hospitals.

In light of these considerations, this study was conducted to investigate the presence of causative agents of IFDs in hospital environments. In addition, susceptibility testing was performed on both antifungal drugs and disinfectants to provide insights into effective treatment and preventive measures. Finally, we compared isolates of clinical cases of fungal infections and their resistance patterns with environmental isolates to identify potential relationships and inform better infection control strategies.

Methods

This study was conducted from May 2022 to November 2022 across seven hospitals affiliated with Isfahan University of Medical Sciences, Iran. A total of 47 air samples and 36 surface samples from the exhaust vent surface of HVAC (Heating, Ventilation, and Air Conditioning) systems were collected in the intensive care units (ICUs) of these hospitals. During the sampling period, patients, staff, and patient attendants were present, while visitors were limited. Temperature and relative humidity were recorded at the time of sampling.

To assess the influence of various factors on the environmental prevalence of causative fungal agents of HAIs, the Targeted Environmental Investigation checklist, developed by the CDC (https://www.cdc.gov/fungal), was utilized. According to this checklist, during the sampling period, the condition of ICU environment such as air infiltration from the open space or other nearby spaces (sealing of windows and non-closing of doors), maintenance and repair of HAVC (replacing filters and cleaning air ducts), environmental cleaning processes (daily preparation of cleaning solutions, daily cleaning of rooms, preparation of disinfectants and disinfection according to instructions) and construction, renovation, demolition and repair activities (internal and external construction) were noted.

Sampling

For air sampling, an all-glass impinger (AGI) containing phosphate buffer saline (PBS) was used. To simulate the respiratory zone, sampling was done at a height of 1.5 m above from the ground level and approximately 2400 L of air were collected using a portable pump during 4 h from each ICU.

Surface sampling of exhaust vents was done by swabbing with sterile Dacron swabs pre-wetted in PBS. The swabs were then placed in tubes containing 3 to 5 mL of PBS. The samples were stored in a cold box and immediately transferred to the laboratory for microbial analysis.

Fungi detection

To detect fungal aerosols, aliquots of each impinger collection medium were plated onto Sabouraud dextrose agar (SDA) (Merck, Darmstadt, Germany) plates containing chloramphenicol after vigorous shaking. To detach cells from swabs, surface samples were vortexed and subjected to three minutes of ultrasonic vibration. Then, duplicate aliquots of the surface sample suspensions were cultured on SDA plates.

All SDA plates were incubated at 25 °C for 3–7 days and examined for fungal growth every 24 h. Subcultures were prepared from each colony to obtain pure cultures. Fungal isolates were identified at the genus level by macroscopic and microscopic characteristics. Given the differences in pathogenic potential and susceptibility profiles of *Aspergillus* species and Mucorales, these fungi, along with unidentified isolates, were further identified at the species level using molecular methods. The airborne fungal colonies were enumerated and quantified in terms of colony forming units per cubic meter (CFU/m^3).

Molecular identification of isolated fungi

DNA of fungal isolates was extracted and purified by adding lysis buffer and glass beads, followed by the phenol-chloroform extraction method as previously described [18]. Extracted DNA was subjected to polymerase chain reaction (PCR) to amplify a fragment of the ITS region using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers. To identify *Aspergillus* species, a fragment of the β-tubulin gene was amplified using Bt2a (5′-GGTAACCAAATC GGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTG TAGTGACCCTTGGC-3′) primers. After PCR, DNA sequencing of the amplified genes was performed, and the sequences were analyzed using the BLAST algorithm with databases from the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence data were deposited into GenBank under accession numbers: PP923758-PP923775.

Susceptibility analysis to antifungal drugs

Antifungal susceptibility testing (AFST) of fungal isolates was performed following the Clinical and Laboratory Standards Institute (CLSI) guidelines for the filamentous fungi (CLSI M38-A2) [19]. The antifungal activity of Itraconazole (ITC), Amphotericin B (AmB), Voriconazole (VRC), and Caspofungin (CAS) (all from Sigma-Aldrich, USA), was tested against *Aspergillus* isolates. For Mucorales, the antifungal activity of Itraconazole, Amphotericin B, Isavuconazole (ISC) and Posaconazole (PSC) (all from Sigma-Aldrich, USA) was evaluated. For testing antifungal activity, all drugs except Caspofungin were used in a concentration range of 0.0312–16 µg/mL and the minimum inhibitory concentration (MIC) was determined. Caspofungin was used at concentrations of 0.0156–8 µg/mL and the minimum effective concentration (MEC) required to inhibit hyphal growth was reported. Two quality control (QC) isolates, *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019, were included in all experiments. Due to the lack of established clinical breakpoints for molds, except for *Aspergillus fumigatus* against ITC, epidemiological cut-off values (ECVs) were used to distinguish drug-susceptible wild-type (WT) strains from those with probable acquired resistance mechanisms [20]. The recommended ECVs for *Aspergillus* species and *Rhizopus arrhizus* are shown in Table S1 (Supplementary file).

Analysis of antifungal efficacy of disinfection agents

Fungal spores in the air tend to settle on surfaces, walls and floors due to the high aerodynamic diameter and consequently may grow on surfaces, especially humid ones. Therefore, surface disinfection is essential to control the growth of filamentous fungi and the spread of their spores [21]. In this study, Kirby-Bauer disc diffusion method was used to evaluate the inhibitory effect of nine commonly used hospital disinfectants on *Aspergillus* spp. and Mucorales. The disinfecting agents tested included sodium hypochlorite at concentrations of 1% and 2% (SH 1%, 2%), hydrogen peroxide (HP), and several commercial disinfectants: SeptiSurface (SS), SeptiTurbo (ST), Isept (IS), VentiSept (VS), CyaSept HI (SHI) and CyaSept HP (SHP). A detailed description of the disinfectants is provided in the supplementary file (Table S2). Briefly, fungal inoculum suspensions were prepared in sterile saline containing 0.01% (v/v) Tween 20, with conidia or sporangiospore concentrations adjusted to approximately 0.5 McFarland standards. Then 200 µL of the homogenous suspension was spread onto SDA plates.

Discs (6 mm in diameter, Whatman filter paper) containing the disinfectant agents were placed on the inoculated plates, which were then incubated at 25 °C for 24–48 h. All experiments were performed in duplicate, and the average inhibition zone diameter for each isolate

was recorded. Sterile distilled water served as the negative control, while phenol was used as the positive control. Sensitivity results were categorized based on the diameter of the inhibition zones as follows: Resistant (≤ 14 mm), Intermediate (15–19 mm), and Susceptible (≥ 20 mm) [22, 23].

Clinical cases

Culturable clinical isolates of *Aspergillus* spp. and Mucorales were obtained from the Fungi Laboratory affiliated with Isfahan University of Medical Sciences between May 2022 and November 2022, a period that partially overlapped with the COVID-19 pandemic. This laboratory received clinical samples from patients admitted to the ICUs of seven surveyed hospitals, where invasive fungal infections were suspected. To enhance clarity and explore potential relationships between clinical and environmental isolates, clinical isolates were further identified through sequencing analysis, and their antifungal resistance profiles were evaluated.

Results and discussion

Fungi isolated in the ICUs environment

Monitoring and controlling bioaerosols is a key strategy for infection prevention in hospital settings. Figure 1 illustrates the distribution of fungal species isolated from the air. This study revealed that 45% (21/47) of air samples collected from the ICUs across various hospitals tested positive for fungal species, with an average concentration of 23 ± 16 CFU/m^3. Comparatively, a Spanish hospital study reported an average fungal concentration of 14 CFU/m^3 [24]. The predominant fungal species identified in the samples were *Aspergillus* spp. (38%) and dematiaceous fungi (36%), including *Cladosporium*, *Alternaria*, and *Curvularia*. Other detected fungal species included *Candida albicans*, *Penicillium* spp., *Gymnascella dankaliensis*, and *Hamigera insecticola*. As shown in Fig. 1, both *Aspergillus* spp. (38%) and dematiaceous fungi or one of these fungal species were present in air of all hospitals, except hospital NO. 5 where no fungi were detected during air sampling. Previous researches have similarly reported *Cladosporium* and *Aspergillus* as dominant species in hospital air. A study by Ghazanfari et al. (2022) found *Aspergillus* (39.5%) and *Cladosporium* (16.6%) to be the most commonly isolated fungi in air samples collected from various wards in 23 hospitals across 18 provinces in Iran [16]. Additionally, Sham et al. (2021) noted that *Aspergillus*, *Cladosporium*, and *Penicillium* spp. were among the most frequently isolated fungal species in hospitals worldwide [25]. The frequent detection of *Aspergillus*, dematiaceous fungi, and *Penicillium* in the hospital air can be attributed to their robust growth on various substrates under diverse weather conditions, coupled with their high spore production and dispersal capabilities [26, 27].

Figure 2 depicts the frequency of fungal species isolated from exhaust vent surfaces. Fungi were present in all collected samples (100%) from the vents. The most prevalent species were *Aspergillus* spp. (55%), dematiaceous fungi (21%), and *Rhizopus* spp. (6%). Additional fungal species identified included members of the *Chaetomium*, *Pyronema*, and *Penicillium* genera. Notably, *A. niger* was the most frequent species (26%), found in samples from multiple hospitals. A study by Azimi et al. (2013) on hospital air-conditioning systems in Iran identified *Aspergillus*, *Penicillium*, and dematiaceous fungi as the dominant species [28]. Similarly, Kelkar et al. (2005) found *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus* to be prevalent in Indian hospital air- conditioning systems [29]. Our findings indicate that exhaust vents provide conducive environments for mold growth and sporulation, making

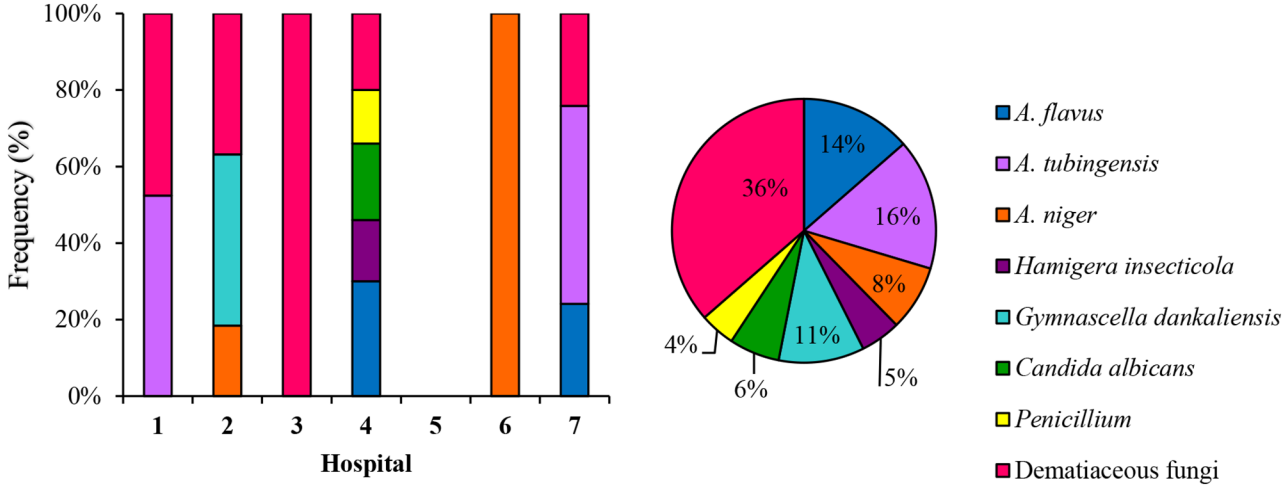


Fig. 1 Frequency of fungal species detected in air samples

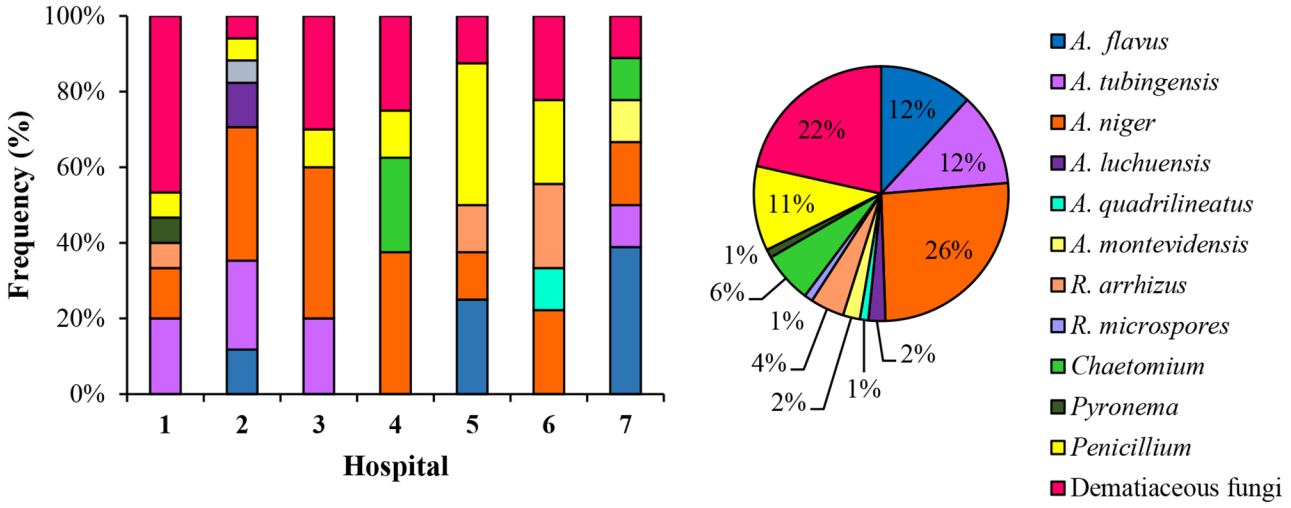


Fig. 2 Frequency of fungal species detected in exhaust vent samples

them significant sources of fungal contamination in hospital settings.

Figures 1 and 2 illustrate the presence of *Aspergillus* spp. and Mucorales in hospital environments, underscoring their potential role in the development of IFDs. Of the over 180 *Aspergillus* species, approximately 20 are recognized as opportunistic pathogens in humans. *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* are responsible for the majority of invasive aspergillosis (IA) cases, with *A. fumigatus* being the primary agent, capable of causing disease at concentrations as low as 1 CFU/m^3 of air [26, 30]. Although *A. fumigatus* was not detected in the hospital environments studied, *A. flavus* and *A. niger* were frequently isolated from both air and surface samples, with concentrations ranging from 6 to 15 CFU/m^3 (Figs. 1 and 2). In a study by Ghazanfari et al. (2022) conducted in 23 hospitals across 18 provinces of Iran, *A. flavus* complex (38/96, 39.6%) and *A. niger* complex (31/96, 32.3%) were the most dominant species isolated from air and equipment [16]. The high prevalence of *A. flavus* and *A. niger* in our study, as well as in other clinical and environmental samples from Iran, is likely due to their high adaptability to hot and dry climates [16]. Notably, *A. flavus* has been reported as the most common etiological agent of invasive aspergillosis in Iran [14].

Rhizopus, a mold genus from the Mucorales order, is most commonly associated with mucormycosis, with *R. arrhizus* accounting for nearly 60% of human cases [31]. As depicted in Fig. 2, R. *arrhizus* was isolated in exhaust vent samples, posing a potential risk to immunocompromised patients in ICUs.

Environmental investigation and potential impact on fungal concentration

Environmental monitoring for the presence of fungi, alongside the assessment of environmental parameters, can reveal potential shortcomings in infection prevention and highlight areas for improvement. Fungi, a prevalent microbial contaminant in indoor settings, have a strong capacity to proliferate on various construction materials, releasing spores that may lead to fungal infections in vulnerable patients [32]. Key factors such as temperature, relative humidity, building materials, air exchange rates, ventilation systems, and construction practices can influence fungal concentrations in hospital environments. Table 1 outlines important environmental parameters assessed using the CDC checklist. In this study, the age of hospital structures was a recurring issue, prompting renovations in some cases. Older buildings and the materials used in construction can contribute to fungal colonization [33]. Research shows that aged and deteriorating buildings exhibit higher levels of fungal contamination compared to newer constructions [34]. The most significant fungal contamination was observed in Hospital No. 4, where concentrations reached 50 CFU/m^3. It is likely that construction activities, such as dust generation, contributed to these elevated fungal levels in ICU air [33]. Park et al. (2019) observed that during construction phases, including demolition and excavation, airborne fungal spore counts increased, correlating with a significant rise in invasive *Aspergillus* infections [4]. In hospitals 1, 2, and 7, fungal concentrations were measured at 31, 38, and 29 CFU/m^3, respectively. Factors such as open ICU windows and the hospitals' proximity to major roads may have contributed to the elevated fungal presence in these facilities. The Centers for Disease Control and Prevention (CDC) recommends implementing control measures such as sealing windows and using special ventilation systems to manage infection risk [35]. The study also revealed a lack of cleaning in the hospitals' exhaust vents, as evidenced by the substantial fungal contamination within these systems. Given their propensity to

Table 1 Important environmental parameters checked by the CDC checklist

Hospital No.	No. of ICUs	No. of Beds	Number of occupants	Ventilation system	Concentration of airborne fungi (CFU/m^3)	Air penetration from outdoors	External or Internal construction	exhaust vents maintenance and repair	Construction or Renovation year
1	4	25	15–20	Central operation HVAC	31	Yes	No	No	1996
2	3	11	8–10	Central operation HVAC	38	Yes	No	No	2002
3	1	8	6–8	Central operation HVAC	18	No	No	No	1978
4	2	15	12–15	Central operation HVAC	50	Yes	Yes (internal)	No	1978
5	3	10	8–10	Central operation HVAC	0	No	No	No	1987
6	4	8	8–10	Central operation HVAC	6	No	No	No	2009
7	3	10	10–14	Central operation HVAC	29	Yes	No	No	1953

HVAC: heating, ventilating and air-conditioning systems

accumulate dust and moisture, these systems are potential hotspots for microbial contamination, necessitating regular cleaning and maintenance to mitigate the spread of pathogenic microbes.

Both temperature and relative humidity are critical factors affecting bioaerosol concentrations, including fungi, in the air and on surfaces [30, 36]. Throughout the study, ICU ambient temperatures ranged from 19 to 27 °C, averaging 21.7 °C, while relative humidity spanned 11 to 39%, with an average of 21.9%. Statistical analysis indicated that relative humidity significantly impacts fungal concentrations, while temperature did not show a notable correlation with fungal counts. Mirhoseini et al. (2015) also reported a significant positive association between relative humidity and bioaerosol numbers in hospital settings, but found no substantial link between temperature and fungal quantities [37]. Notably, despite Isfahan's semi-arid climate and low humidity levels, which are generally unfavorable for fungal growth, *Aspergillus* species, being xerophilic, can thrive and produce spores even in environments with low atmospheric relative humidity and on substrates with minimal moisture content [38].

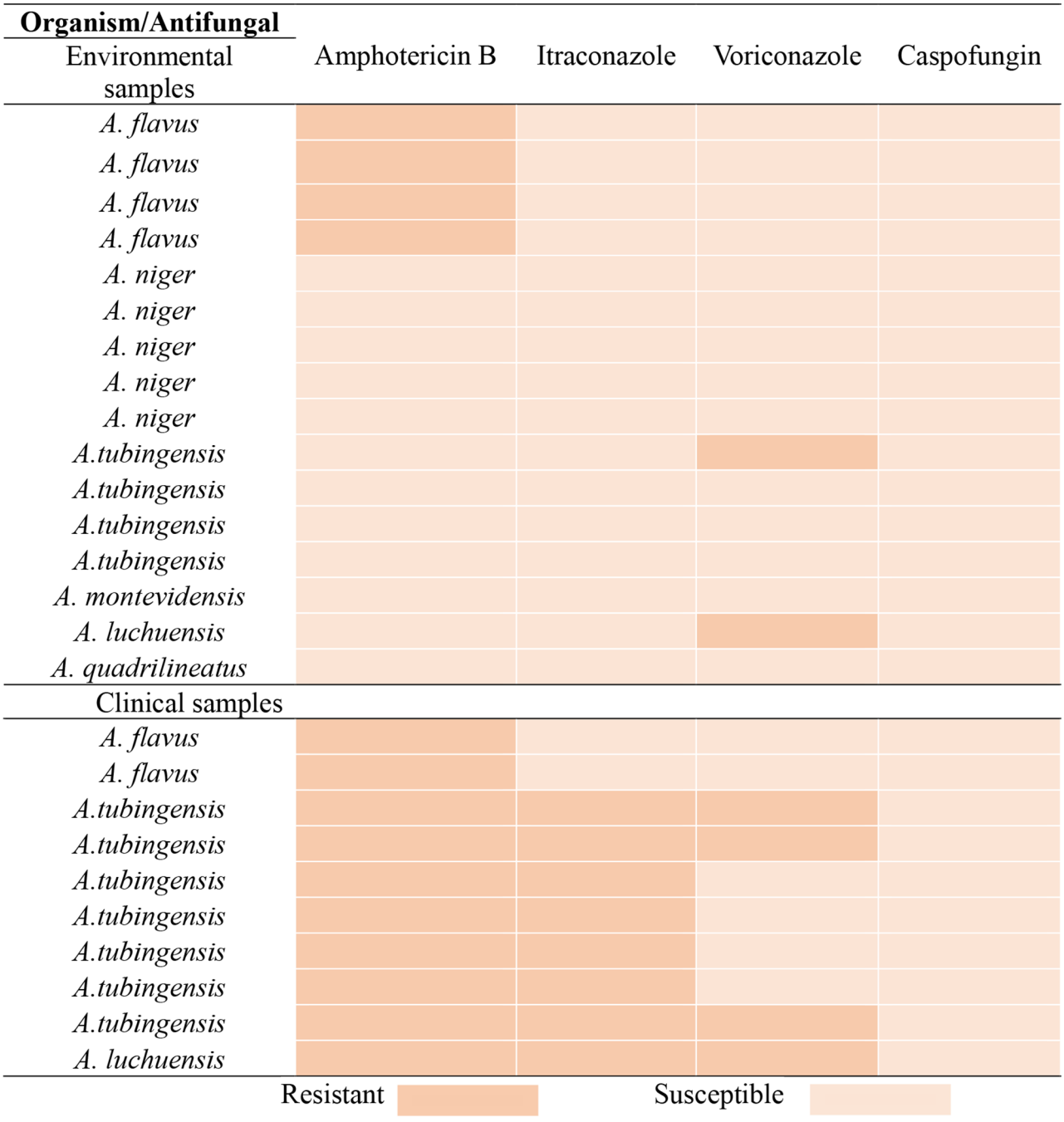
Antifungal drug resistance in isolated fungi

The increasing resistance of pathogenic fungi to antifungal drugs presents a growing challenge to healthcare systems. The widespread use of antifungal medications has altered the epidemiological profile of fungal infections, with a rise in drug-resistant fungal species in clinical settings, often leading to treatment failures. As hospital environments contaminated with fungal spores are a source of nosocomial fungal infections, analyzing the antifungal susceptibility profiles of isolated species can enhance antifungal stewardship efforts [39].

Table 2 presents the susceptibility profile of *Aspergillus* spp. to tested antifungal drugs based on the obtained MICs and the proposed ECVs for *Aspergillus* spp. (Supplementary file; Table S3, S5, S6, S7). Notably, 25% (4/16) of *Aspergillus* isolates exhibited a MIC above the ECV of 2 µg/mL recommended for *A. fumigatus* when tested against AmB. Alarmingly, all *A. flavus* isolates from the hospital environment had an MIC of >2 µg/mL against AmB, indicating a concerning trend of emerging drug resistance among *A. flavus*. This finding aligns with the results of Moslem et al. (2020), who also reported resistance to AmB in environmental *A. flavus* isolates [40]. Additionally, one isolate each of *A. tubingensis* and *A. luchuensis* showed resistance to VRC (MIC>1 µg/mL). All other species demonstrated drug sensitivity. Our results also showed no resistance of *Aspergillus* spp. to ITC and CAS (Table 2). Consistent with our findings, a study on *Aspergillus* spp. isolated from hospital surfaces and equipment in Iran showed no resistance in *A. flavus* isolates to VRC and ITC. In contrast, about half of *A. fumigatus* isolates had an MIC≥2 µg/mL against VRC and ITC and some strains of *A. tubingensis*, *A. luchuensis*, and *A. niger* were resistant to ITC [14]. Ghazanfari et al. (2023) reported that, of the nine *A. tubingensis* isolates obtained from environmental samples (instruments and air) from different wards in four educational hospitals in Mazandaran Province, Iran, 22.2% and 44.4% exhibited an MIC of ≥2 µg/mL against VRC and ITC, respectively [15]. Manharpreet Kaur et al. (2024) found that 5% (3/62) fungal isolates from hospital environments had an MIC of >2 µg/mL for AmB [24]. Monpierre et al. (2021) observed that only two out of 51 environmental *Aspergillus* isolates were azoles-resistant [41]. Overall, the azole resistance in hospital environmental isolates of *Aspergillus*, highlights a threat to patients, who are vulnerable to invasive aspergillosis.

Table 3 demonstrate the susceptibility profile of *Rhizopus* spp. to tested antifungal drugs based on the obtained MICs and proposed ECVs for *R. arrhizus* (Supplementary file; Table S3, S4, S6, S8). According to the MICs of tested antifungal drugs for *Rhizopus* spp., 25% (1/4) of *Rhizopus* isolates from environmental samples were resistant to AmB, with MIC values exceeding the ECV for *R. arrhizus* (Table S3). This isolate also showed resistance to ISC and PSC while no resistance was detected against ITC according to ECVs recommended for *R. arrhizus* [42].

Table 2 Susceptibility profile of *Aspergillus* spp. to tested antifungal drugs (resistance indicates the isolates with MIC greater than the ECV proposed for *Aspergillus* spp. against the specific antifungal drug)



Efficacy of disinfectants against fungal isolates

The misuse or inappropriate concentration of disinfectants can lead to suboptimal decontamination in hospital environments, posing a health risk to patients by allowing the persistence of opportunistic or pathogenic microorganisms. Recent studies have indicated that continuous exposure to sublethal concentrations of disinfectants can lead to microbial resistance, and in some cases, co- or cross-resistance to antimicrobial drugs [43]. Table 4 details the effects of nine disinfectants on *Aspergillus* spp. and Mucorales isolates from air and exhaust vent surfaces across various hospitals. The fungi exhibited a broad sensitivity spectrum, with some isolates showing no inhibitory zone and others displaying zones up to 26 mm. Resistance to disinfectants ranged from 43.5 to 73.9%, with IS and SHI showing the highest and lowest resistance, respectively. Figure 3 illustrates

Table 3 Susceptibility profile of *Rhizopus* spp. to tested antifungal drugs (resistance indicates the isolates with MIC greater than the ECV proposed for *Rhizopus* spp. against the specific antifungal drug)

Organism/Antifungal	Amphotericin B	Itraconazole	Posaconazole	Isavuconazole*
Environmental samples				
R. arrhizus				
R. arrhizus				
R. arrhizus				
R. microsporus				
Clinical samples				
R. arrhizus				
R. arrhizus				
R. arrhizus				
R. arrhizus				
R. microsporus				

*MIC of *Rhizopus* spp. compared with ECV proposed for *A. Fumigatus*.

Resistant Susceptible

Table 4 Sensitivity analysis of fungal species isolated from the environmental samples (frequency) to common disinfectants

Type of Disinfectant	Sensitive isolates (%)	Intermediate isolates (%)	Resistant isolates (%)
SS	17.4	34.8	47.8
ST	17.4	34.8	47.8
VS	4.4	47.8	47.8
IS	-	26.1	73.9
SH 1%	-	34.8	65.2
SH 2%	21.8	26.1	52.1
SHI	17.4	39.1	43.5
SHP	13	34.7	52.3
HP	17.4	26.1	56.5

that *Aspergillus* spp. had high resistance to many disinfectants, while *Rhizopus* spp. were resistant to all tested agents. These findings underscore the need for further research into disinfectants capable of effectively eradicating fungal spores.

Interestingly, isolates of the same species collected from different environments (air and exhaust vents) responded differently to disinfectants. For example, *A. flavus* and *A. tubingensis* from air samples were relatively sensitive to most disinfectants, whereas their counterparts from exhaust vents exhibited greater resistance. This increased resistance among exhaust vent isolates may be attributed to prior disinfectant exposure. The CDC reports that environmental *Aspergillus* can develop resistance to antifungal drugs following exposure to fungicides similar to medical antifungals, raising significant concerns about patient inhalation of these resistant spores. Consequently, it is crucial to employ disinfectants that effectively eliminate fungi on surfaces, as exposure to sublethal fungicide concentrations may induce antimicrobial resistance.

Network analysis of simultaneous resistance in *Aspergillus* spp. and *rhizopus* spp. to antifungal drugs and disinfectants

The presence of fungal pathogens in hospital environments, coupled with their simultaneous resistance to both antifungal drugs and disinfectant agents, raises significant concerns for the care of vulnerable patients. As depicted in Fig. 3a, *Aspergillus* spp. exhibited the highest resistance (25%) to AmB, with 100% of the species also showing resistance to SHI, SHP, and HP disinfectants. Notably, *Aspergillus* spp. did not exhibit resistance to CAS and ITC. Furthermore, 37.5% of isolates resistant to disinfectants did not show resistance to antifungal drugs, suggesting that, fortunately, these species are not yet drug-resistant. Additionally, Fig. 3b shows that one isolate of *R. arrhizus* which was simultaneously resistant to AmB, ISC, and PSC also demonstrated resistant to all tested disinfectants, highlighting the simultaneous resistance of Mucorales to both antifungal drugs and disinfectants. The simultaneous resistance of *Aspergillus* spp. and Mucorales to antifungal drugs and disinfectants may pose a significant challenge in controlling IFDs in hospital environments, necessitating further research in the future.

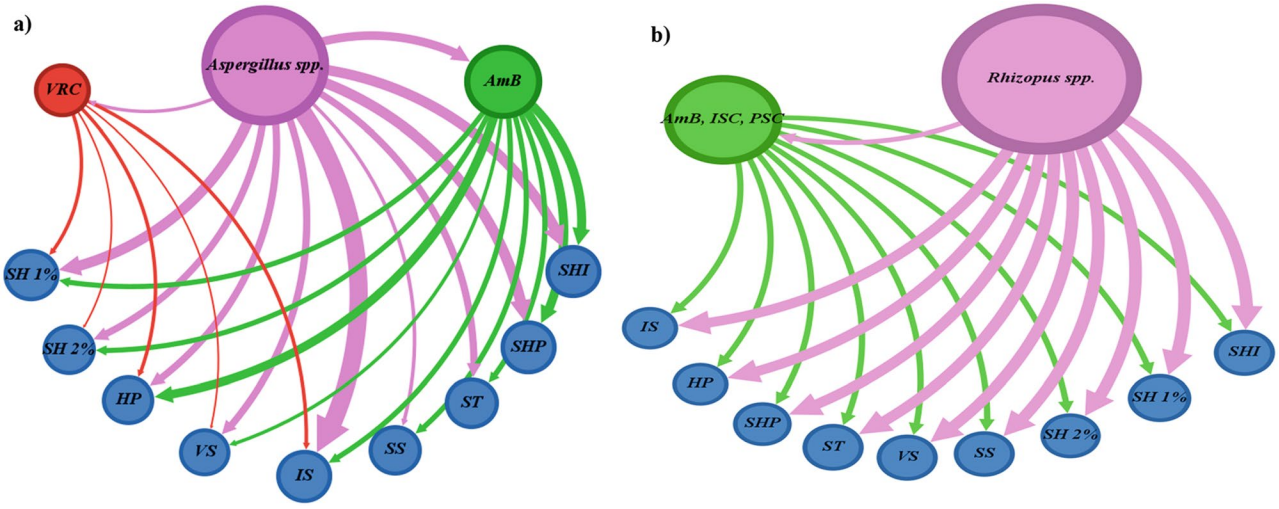


Fig. 3 Network analysis of simultaneous resistance to antifungal drugs and disinfectants of environmental isolates of **a**) *Aspergillus* spp. **b**) *Rhizopus* spp. (The size of nodes and the width of edges is related to the relative abundance of resistant fungi)

Clinical cases of aspergillosis and mucormycosis

Investigating the sources of aspergillosis and mucormycosis infections in healthcare settings is particularly challenging due to patients' complex medical histories and the uncertain incubation period of these IFDs [43, 44]. Despite these challenges, the significance of nosocomial mycoses led us to investigate the potential role of hospital environment in acquiring of fungal infections. We compared environmental data with clinical cases in two hospitals (Hospital No. 1 and Hospital No. 5), which had the highest reports of invasive aspergillosis and mucormycosis cases. As detailed in Table S9, 23 cases were identified, with the majority occurring in Hospital No. 1. Notably, the species of *Aspergillus* and Mucorales most frequently identified in clinical cases mirrored those found in the environmental isolates (Tables 2 and 3, and S9). This similarity may indicate that the hospital environment could be a potential source of nosocomial fungal infections.

In environmental microbiology, antimicrobial profiling is commonly used as a phenotypic method for microbial source tracking [39]. It has been reported a rise in azole-resistant *Aspergillus fumigatus* among patients, reflecting an increase in azole resistance in environmental isolates [45]. Snelders et al. (2009) suggested that since inhalation of airborne *Aspergillus* spores is the common route of infection, the dominance of a single resistance mechanism in clinical azole-resistant isolates may indicate acquisition from a common environmental source [46]. Therefore, we compared the antifungal sensitivity profiles of similar species isolated from both the environment and clinical samples (Tables 2 and 3).

The antifungal susceptibility profile of clinical isolates against AmB showed that 100% of *Aspergillus* spp. had MIC higher than ECV for *A. fumigatus*, indicating resistance (Table 2). A review by Fakhim et al. (2022) reported that approximately 25% of clinical *Aspergillus* spp. are resistant to AmB [47]. Our results also indicated that all *Rhizopus* spp. isolates are resistant to ISC (Table 3). Notably, all clinical *A. flavus* isolates exhibited resistance to AmB, consistent with the antimicrobial resistance pattern of *A. flavus* isolates from environmental samples. Furthermore, two *R. arrhizus* isolates from clinical samples shared an antimicrobial profile with an *R. arrhizus* environmental isolate, showing resistance to both AmB and ISC.

Although our findings revealed more resistance in clinical isolates, the comparable antifungal resistance profiles of clinical and environmental isolates of *A. flavus* and *R. arrhizus* underscores the potential role of the hospital environment as a source of nosocomial fungal infections. It is noteworthy that at least two-thirds of patients with azole-resistant infections have not received prior azole therapy, suggesting an environmental route of acquisition [45]. Snelders et al. (2009) provided evidence that patients with invasive aspergillosis due to azole-resistant *A. fumigatus* likely acquired the fungus from the environment [46]. In another study of four cases of mycotic endocarditis among open-heart surgery patients within a single year, including one case involving *Mucor* sp., investigators discovered *Mucor* sp. and *Aspergillus* sp. in dust from an air conditioner duct and in air samples. They concluded that the air filter in use was likely insufficient to trap spores [44].

Mitigating the risk of transmission of fungal spores

To mitigate the risk of nosocomial transmission of *Aspergillus* and Mucorales through hospital environments, it is imperative to implement infection control measures swiftly to curb their spread in healthcare settings. Contaminated surfaces with fungal growth may be

a significant source for the release of fungal spores and subsequent acquisition of fungal infections. Therefore, healthcare facilities must follow strict cleaning protocols, especially for humid surfaces such as exhaust vents.

A major challenge in clinical wards is preventing the entry of fungi that are ubiquitous outdoors. By keeping windows closed and employing high-efficiency particulate air (HEPA) filters in areas housing high-risk patients, it is feasible to improve air quality within clinical units.

During the disinfection process of hospital environments, it is crucial to assess both the efficacy and appropriate concentration of disinfectants to combat opportunistic and pathogenic microorganisms. This evaluation is particularly vital for controlling resistance mechanisms, which may develop due to repeated exposure to sub-lethal concentrations of antimicrobial agents.

Since, transmission-based precautions are a critical component in the prevention and control of airborne pathogens, such as *Aspergillus* and Mucorales [48], proactive environmental surveillance to identify potential sources of fungal spores before they lead to infections is a key strategy in high-risk wards. Regular monitoring, combined with appropriate infection control measures, can help to minimize the risk of fungal transmission in healthcare settings.

Conclusions

Our study indicates that hospital environments, particularly exhaust vents, can serve as significant reservoirs for the dissemination of causative agents of IFDs, such as *Aspergillus* and *Rhizopus*. The antimicrobial analysis revealed that some environmental isolates exhibit resistance to antifungal drugs. Furthermore, common disinfectants currently employed in hospitals are ineffective at inactivating fungal spores. The challenge of managing these spores is further complicated when they demonstrate simultaneous resistance to both antifungal drugs and disinfectants. Given the difficulties in treating IFDs, identifying and controlling the sources of mold within healthcare facilities is crucial to ensure a safe environment for patients. Additionally, our findings underscore the urgent need for the development of more potent disinfectants capable of effectively eradicating fungal spores, thereby reducing the risk of nosocomial fungal infections. Further research is also necessary to fully understand the precise mechanisms of co-selection of fungal spores to antifungal agents.

Abbreviations

IFDs	invasive fungal diseases
ICU	Intensive care units
HAIs	Hospital-acquired infections
HVAC	Heating, Ventilation, and Air Conditioning
AGI	All-glass impinger
PBS	Phosphate buffer saline
SDA	Sabouraud dextrose agar
PCR	Polymerase chain reaction
AFST	Antifungal susceptibility test
CLSI	Clinical and Laboratory Standards Institute
ITC	Itraconazole
AmB	Amphotericin B
VRC	Voriconazole
CAS	Caspofungin
ISC	Isavuconazole
PSC	Posaconazole
MIC	Minimum inhibitory concentration
MEC	Minimum effective concentration
QC	Quality control
ECVs	Epidemiological Cut-off values
SH 1%, 2%	Hypochlorite 1%, 2%
HP	Hydrogen peroxide
SS	SeptiSurface
ST	SeptiTurbo
IS	Isept
VS	VentiSept
SHI	CyaSept HI
SHP	CyaSept HP

Supplementary Information

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Supplementary Material 1

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Author contributions

Soudabeh Ghodsi: Investigation, Methodology, Data curation, Data analysis, Writing; Mahnaz Nikaeen: Conceptualization, Funding acquisition, Supervision, Writing, Reviewing, Editing; Shima Aboutalebian: Investigation; Rasoul Mohammadi: Validation; Hossein Mirhendi: Supervision.All authors reviewed and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

We carried out this study according to the Vice-Chancellor for research Affairs-Medical University of Isfahan with ethic code of IR.MUI.RESEARCH.REC.1400.477.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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