ISSN: 2588-4115; Infection Epidemiology and Microbiology. 2024;10(1):43-50 doi 10.52547/iem.10.1.43

Surveillance of Fungal Airborne Contamination in Hospital Wards in Indonesia 2020-2021: Impact of HEPA Filters and Occupancy

ARTICLE INFO

Article Type
Original Article

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How to cite this article
Helmi-Aziz M., Tjampakasari C.R., Aditianingsih D., Wahid M. Surveillance of Fungal Airborne Contamination in Hospital Wards in Indonesia 2020-2021: Impact of HEPA Filters and Occupancy. Infection Epidemiology and Microbiology. 2024;10(1): 43-50.

Article History
Received: September 16, 2023
Accepted: February 17, 2024
Published: February 21, 2024

ABSTRACT

Background: Airborne biological agents in hospitals, such as fungal micro-colonies, play a significant role in life-threatening airborne infections in immunocompromised individuals. Thus, it is crucial to reduce airborne contamination and address the related influencing factors. This study aimed to evaluate indoor air quality (IAQ) in terms of fungal contamination and factors that could influence IAQ in hospital rooms. .

Materials & Methods: This environmental surveillance study was conducted in two rooms for one year, and 288 air specimens were collected using the active air sampling method equipped with chloramphenicol-supplemented Sabouraud dextrose agar. Temperature, relative humidity, and occupants' number were also recorded. Fungal colony counts were recorded and converted using the Feller table. Furthermore, fungi were identified based on macroscopic and microscopic characteristics.

Findings: The mean difference of isolated fungi between the two rooms was statistically significant ($p < .0001$). Yeast, *Penicillium* spp, and *Aspergillus* spp. were the most predominant fungi. Both rooms had temperature and relative humidity above the national recommended levels (above 23 ˚C and %60). Occupants' number in the room without HEPA filter was significantly correlated with airborne fungal contamination level.

Conclusion: The level of airborne fungal contamination was significantly higher in the room without a HEPA filter. Yeast, *Aspergillus* spp., and *Penicillium* spp. were the most predominant fungi isolated from both rooms. Room temperature and relative humidity had no effect on airborne fungal contamination level. Occupants' number in the room without HEPA filter influenced airborne fungal contamination level.

Keywords: Fungal count, Air filters, Air pollutants, Nosocomial infection

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Introduction

Airborne biological agents in hospitals play a significant role in the incidence of airborne nosocomial diseases. Fungi, as airborne biological agents in hospitals, could grow and produce micro-colonies inside hospital rooms, especially during months when the temperature and humidity are high, and the air is not conditioned [1-3]. Those fungal micro-colonies in the form of spores, mycelia, and hypha fragments could be inhaled and cause adverse health effects, particularly in immunocompromised individuals such as patients treated in hematology wards or intensive care units [4]. These adverse health effects range from allergic reactions to life-threatening respiratory infections in immunocompromised individuals [5, 6].

In order to reduce hospital airborne fungal contamination, several factors should be controlled. Several factors that have been proven to contribute to the amount of airborne fungal contamination are the use of HEPA filters, room temperature, relative humidity, and the presence of occupants in the dedicated room [5, 7-9]. Thus, understanding the profile and reduction of airborne nosocomial fungal contamination is one of the best approaches to reduce the incidence of nosocomial adverse effects caused by fungi in immunocompromised individuals.

Objectives: The current study aimed to evaluate indoor air quality (IAQ) in terms of fungal contamination and factors that could influence IAQ in hospital rooms occupied by immunocompromised patients. This study also aimed to evaluate the fungal genera circulating in the rooms occupied by immunocompromised patients.

Materials and Methods

This environmental surveillance study was performed using the active air sampling method in order to determine the indoor air quality of the hematology ward, located on the 8th floor of the new building (room without HEPA filter), and the adult intensive care ward, located on the 2nd floor of the surgery building (room equipped with HEPA filter), at Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. The area of the hematology ward room was 50.16 m^2, and the area of the intensive care ward room was 264.8 m^2. Based on the area of the rooms, 24 air samples were collected every month for one year since October 2020 from eight sampling locations in the hematology ward and 16 sampling locations in the intensive care ward using an air sampler with an aspiration volume of 100 L/min (MAS-100 NT® Microbial Air Sampler, Merck), equipped with a Sabouraud dextrose agar (SDA) plate supplemented with chloramphenicol [1]. Air sampler was placed 1 m away from the walls and 1 m above the floor in each sampling location [1].

During air sample collection, the number of occupants, temperature, and relative humidity of each room were also recorded to determine whether these factors could affect the level of airborne fungal contamination [5]. After collecting the air samples in agar plates, the plates were incubated at 30 ˚C for ten days [1]. After 48 hours of incubation, the number of colonies was counted and converted to colony forming units per cubic meter (CFU/m^3) using the Feller table provided by the air sampler manufacturer [1]. After ten days of incubation, the fungal colonies were identified based on their macroscopic and microscopic morphological characteristics [1]. Slide culture was performed for unidentified fungi to induce sporulation of distinct reproductive structures microscopically.

The data were statistically analyzed using GraphPad Prism software Version 9.0 for Windows (GraphPad Software, San Diego, California, USA). Normality test was performed for all continuous data. The mean

difference of CFU/m^3 between rooms was determined using the Mann-Whitney test. Correlation between variables was analyzed using bivariate Spearman and expressed as Spearman's correlation coefficients (ρ). The calculation was considered statistically significant if the *p*-value was < .05.

Findings

Comparison profile of airborne fungal contamination between the two rooms: During the one-year period of air sample collection, 288 air samples were collected, 96 samples from the hematology ward and 192 samples from the adult intensive care ward. The mean load of fungi isolated from the hematology ward was 273 (±324) CFU/m^3. Meanwhile, in the intensive care ward, the mean load of isolated fungi was 17 (±8) CFU/m^3. The mean difference of isolated fungi between the two rooms was statistically significant (Figure 1) (p< .0001). According to the results, 19 fungal genera were identified among 296 fungal species isolated from the hematology ward. On the other hand, 22 fungal genera were identified among 439 fungal species isolated from the adult intensive care ward. Among these fungal genera, three groups of fungi, including yeast, *Penicillium* spp., and *Aspergillus* spp. were the most predominant fungi isolated from both rooms (Figure 2).

Factors influencing airborne fungal contamination level: The results related to room temperature, relative humidity, and the number of occupants measured during one year are provided in Table 1. The median room temperature in the hematology ward was 24.9 ˚C (22.4–26.5 ˚C), and the median relative humidity was 56% (51–73%). Meanwhile, in the adult intensive care ward, the median room temperature was 22.5 ˚C (21.1–24.6 ˚C), and the median relative humidity was 56% (51–66%). According to the results, both rooms had temperature and relative humidity above the national recommended levels based on the national regulations of the Indonesian Ministry of Health (No. 7/2019) (22-23 ˚C and 40-60% relative humidity).

However, Spearman's correlation showed that the increase in room temperature and relative humidity was not correlated with the amount of airborne fungal contamination

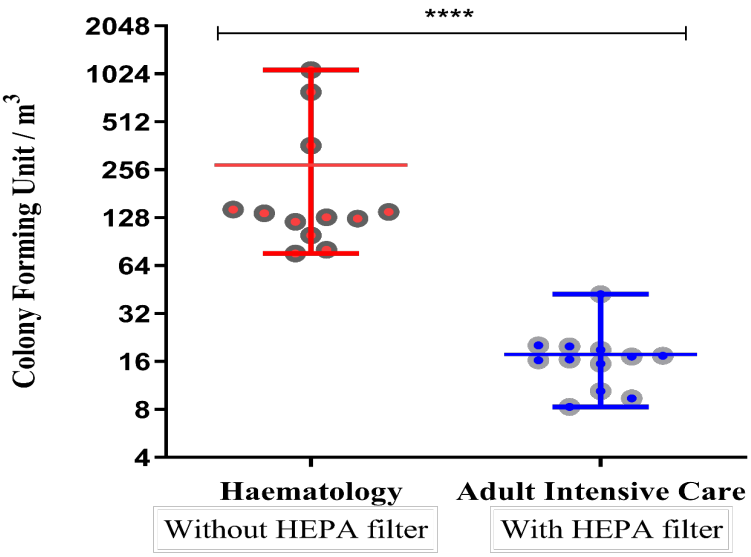

Figure 1) Comparison of mean fungal airborne contamination between the two rooms. HEPA-filtered room (intensive care) has lower concentration of airborne fungi (17 ± 8 CFU/m^3) compared to non-filtered room (hematology) (273 ± 324 CFU/m^3). Comparison was performed using the Mann-Whitney test with p< .0001.

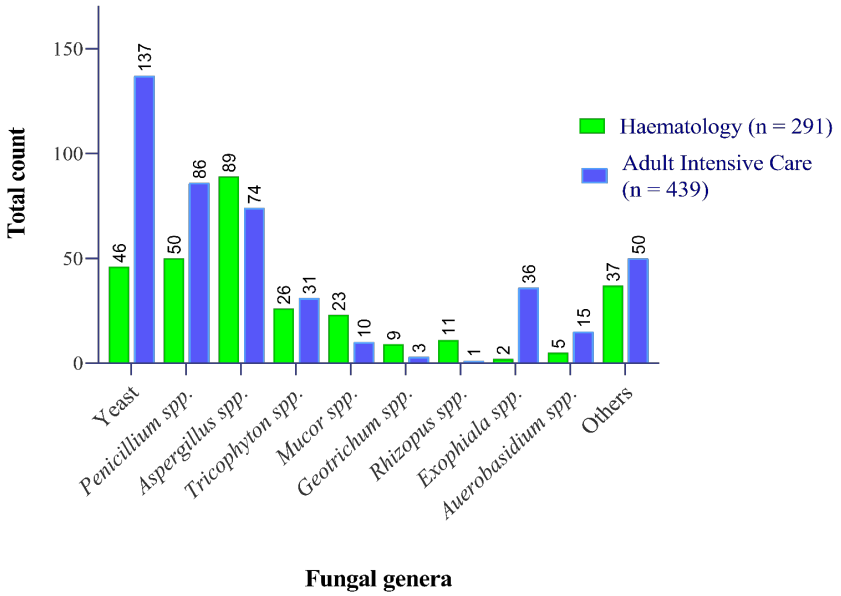

Figure 2) Distribution of fungal genera isolated from both rooms. Yeast, *Penicillium* spp., and *Aspergillus* spp. were the most predominant fungi isolated from both rooms.

Table 1) Measurement results of room temperature (˚C), relative humidity (%), and the number of occupants in hematology ward and intensive care ward.

No	Month	Room Temperature (˚C)		Relative Humidity(%)		Number of Occupants	
		Hematology	Adult IC	Hematology	Adult IC	Hematology	Adult IC
1.	Oct	23,9	22,2	67	62	12	34
2.	Nov	24,8	23,4	64	62	7	37
3.	Dec	25,1	22,9	54	57	7	39
4.	Jan	23,6	23,1	55	64	6	36
5.	Feb	25,1	22,8	61	61	13	20
6.	Mar	24,1	21,6	71	66	16	43
7.	Apr	26	24,6	56	56	10	41
8.	May	25,5	21,2	56	54	8	37
9.	Jun	23,3	21,5	55	54	5	28
10.	Jul	22,4	21,1	51	55	9	19
11.	Aug	25,3	21,7	53	51	6	46
12.	Sep	26,5	23,1	73	55	5	31
	Median	24.9	22,5	56	56	7	36
	Min-Max	22,4 – 26.5	21,1 – 24,6	51 - 73	51 - 66	5 - 16	19 - 46

Notes: IC= intensive care

(p> .05) (Table 2). In addition, in the hematology ward, the room without HEPA filter and the number of occupants were significantly correlated with the amount of airborne fungal contamination.

Table 2) Correlation among three observed variables related to the level of airborne fungal contamination in hematology ward and intensive care ward.

No	Variables	Hematology (Without HEPA Filter)		Adult Intensive Care (With HEPA Filter)	
		P	*P-Value*	*P*	*P-Value*
1.	Room temperature	.08	.79	.21	.49
2.	Relative humidity	.35	.26	.34	.26
3.	Number of occupants	.62	.03	- .02	.94

Discussion

This study aimed to evaluate airborne fungal contamination and several factors that could influence the level of airborne fungal contamination in immunocompromised-occupied rooms. During the one-year study period, it was found that in the hematology ward, the room without a HEPA filter had a significantly higher level of airborne fungal contamination than the room equipped with a HEPA filter in the adult intensive care ward. This result is in line with the results of previous studies showing that a well-functioning central or portable HEPA filter in a hospital room could reduce airborne fungal contamination by up to 70.2% [9-12]. One study also showed that if the HEPA filter was idle for a month, the amount of airborne fungal contamination increased by 132% [10]. Studies have shown that the use of HEPA filters not only enhances indoor air quality but also reduces the prevalence of nosocomial infections of *Aspergillus* spp. among immunocompromised patients [11, 13-15]. However, not only the use of HEPA filters is essential, but also the maintenance of HEPA filters is one of the things that must be taken into account. Studies have mentioned that several microbes could be isolated from HEPA filters that do not undergo maintenance even after 6-12 months of usage [10, 16]. This statement is in line with the finding of this study, as a low level of fungal contamination was observed in HEPA-filtered rooms, where the HEPA filters undergone maintenance only once a year. This finding is crucial since the existence of 0.1 CFU/m^3 of *Aspergillus* spp. spores could cause invasive aspergillosis in immunocompromised patients [17].

Regarding the fungal genera that were successfully isolated, we were able to recover only opportunistic fungi. Yeast, *Aspergillus* spp., and *Penicillium* spp. were the three most predominant fungal groups isolated from both rooms. Similar to previous studies, *Aspergillus* and *Penicillium* spp. were the most predominant fungi circulating in hospital rooms in various locations [1, 5, 18-20]. However, since molecular approach was not performed in this study, it was not possible to identify the isolated fungi at the species level. In addition, the isolated fungi had to be compared with outdoor fungi in order to determine the effect of outdoor contamination on indoor air quality, which was not done in this study. Yeast was the most predominant fungus isolated from the HEPA-filtered room. This finding could be due to the fact that several yeasts, such as *Candida* spp., are normal skin flora that could shed naturally from the skin and be caught during sample collection. This evidence is in line with the results of similar studies successfully isolating *Candida parapsilosis* (*C. parapsilosis*) from hospital air [21-24].

In this study, several factors that could influence the level of airborne fungal contamination were also evaluated. It was observed that the room with a higher median temperature had a higher contamination level than the room with a lower median temperature. However, this

difference was not statistically significant. In addition, there are conflicting results on whether room temperature could influence the level of airborne fungal contamination. Several studies have been able to prove the influence of room temperature. Meanwhile, a study pointed out that a 12 ˚C difference in room temperature did not contribute to the abundance of *Aspergillus* spp. spores inside the hospital room [25-28].

Relative humidity is one of the factors contributing to the growth of airborne biological agents, including fungal spores. The higher the relative humidity level in the room, the higher the adsorption rate of hospital building materials, which becomes a favorable place for bacterial and fungal growth [7].

In this study, it was observed that the relative humidity exceeded the recommended level. However, statistically, relative humidity was not found to influence the level of airborne fungal contamination. Nevertheless, some studies have shown a low correlation between relative humidity and the level of airborne fungal contamination [8, 27, 29].

The number of occupants significantly influenced the level of airborne fungal contamination in the room without a HEPA filter. Despite the fact that the room without a HEPA filter had a lower number of occupants, during air sampling, it was observed that occupants were engaged in activities that contributed to the increased airborne fungal contamination, such as talking, sneezing, coughing, wandering around the hospital, opening the ward door, and flushing toilet [30, 31]. One study also showed that the number of occupants was not correlated with the level of airborne fungal contamination, but the activities they performed were the ones that contributed to the increased airborne fungal contamination [27]. Thus, this finding needs to be evaluated and addressed in future research in order to determine whether the number of occupants significantly influences the level of airborne fungal contamination. To sum up, provision of air-conditioned rooms (air-filtered rooms) and control of environmental factors (temperature, relative humidity, and occupancy) should be done in each hospital room, especially where immunocompromised patients reside.

Conclusion

The level of airborne fungal contamination was significantly higher in the room without a HEPA filter. Yeast, *Aspergillus* spp., and *Penicillium* spp. were the most predominant fungi isolated from both rooms. This airborne fungal assessment may provide crucial information about fungal concentration levels and their implications for nosocomial infection control. In this study, room temperature and relative humidity had no effect on airborne fungal contamination level. However, the number of occupants in the room without a HEPA filter influenced the level of airborne fungal contamination. In addition to airborne fungal assessment, healthcare workers should also address environmental factors that could influence the level of airborne fungal contamination as key points to prevent nosocomial infections.

Acknowledgments

We would like to thank all the laboratory staff and nurses (Lindah, Heti, Ika, Sondang, Dede, Farida, and Cahyo) who helped us in collecting data and completing the study.

Ethical permissions: The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Universitas Indonesia(KET-705/UN2.F1/ETIK/PPM.00.02/2020).

Conflicts of interests: The authors declare no conflict of interest.

Authors' contributions: Main researcher: MHA, CRT, DA, and MW; Methodologist: MHA, CRT, DA, and MW; Data analyzer: MHA,

CRT, DA, and MW; Writer of the introduction and discussion: MHA, CRT, DA, and MW.

Funding: The study was supported by a grant from the Directorate of Research and Development Universitas Indonesia through the International Indexed Publication (Publikasi Terindeks Internasional/PUTI) *Saintekes* 2020 research grant (grant no. NKB-2274/UN2.RST/HKP.05.00/2020).

Consent to participate: Since the study did not involve human subject, we do not perform consent for participation.

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