RESEARCH ARTICLE

Isolation of fungi in indoor air environment of selected air-conditioned and non-air-conditioned wards in a public tertiary hospital in Metro Manila, Philippines

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ABSTRACT

Background and Objectives: The hospital as a health care facility has also become a source of infection that provides a place for different microbiological agents such as fungi. Exposure to these organisms is specifically detrimental to highly immunocompromised in-house patients. This study aimed to 1) detect the presence of fungi in a public tertiary hospital in Metro Manila; 2) determine the dominating fungal organism; and 3) describe the environmental conditions and physical factors affecting the proliferation of fungal organisms.

Methodology: Eight sampling sites were selected for this study. The hospital main lobby was the comparison site for the three non-air-conditioned surgery wards (NACWs) while the fourth level nurse station is the comparison site for the air-conditioned wards (ACWs). Meteorologic conditions such as environmental temperature and relative humidity were also determined. Andersen air sampler was utilized to conduct the environmental indoor air sampling. A total of 98 malt extract agar supplemented with chloramphenicol (0.01%) plates were utilized for the duplicate sampling in eight sites. After three to five days of incubation at 37° C, the isolated fungal organisms were culturally and morphologically characterized.

Results: Seven fungal organisms were isolated from the indoor air sampling conducted namely: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia* sp., *Penicillium* sp., *Alternaria* sp. and *Rhizopus* sp. The most dominant fungal species among the NACWs was *A. niger*. On the other hand, *A. fumigatus* was the most observed isolate among the ACWs. The air-conditioned wards showed a higher number of fungal isolates. In particular, *A. fumigatus* and *A. flavus* colonies in the ACWs were evidently higher than in the NACWs.

Conclusion: The ubiquitous nature of the *Aspergillus* species and slow settling rate due to small spore size make it the most dominant fungal organism retrieved in the air sampling conducted. No strict numerical guidelines were available for the spore counts of *Aspergillus* species to assess contamination rate. However, according to the Health Protection Surveillance Centre, 2018, the values of CFU/m^3 of most of the isolates not only by *Aspergillus* species showed non-compliance with the threshold level documented.

Keywords: *indoor air sampling, ventilation type, nosocomial, Andersen air sampler, relative humidity, temperature*

Introduction

The hospital, as a whole is an integral part of an organizational and medical system that provides comprehensive health services to the community. The hospital as a health care facility has also become a source of infection that provides a place for different microbiological agents, including viruses, bacteria, and fungi. The acquisition of these infections in a medical institution is termed as Health Care Associated Infections (HAIs) [1]. These HAIs have received significant attention in recent decades, and are considered a public health concern.

The routes of transmission of HAIs include airborne transmission, contact transmission, common vectors, contaminated medical devices, and instrumentations. Infection transmitted via the airborne pathway or aerosol transmission is the leading cause of mortality and morbidity worldwide [2]. Moreover, the fungal spores of *Aspergillus fumigatus* and *Penicillium chrysogenum* can cause respiratory infections among the occupants of a hospital. Fungi are among the most successful organisms that are evident in their adaptations in various ecological conditions, primarily due to

their diverse reproductive capacity such as spores, hyphae and sporangia or the fungal propagules that are present in both the outdoor and indoor environments. Fungal infections are evident among immune compromised individuals which are manifested as simple allergies to systemic infections but mycoses can also affect the immune competent ones [3].

Limitations of the current diagnostic tests available to establish an early diagnosis of fungal infection and the emergence of fungal pathogens that are resistant to antifungal agents make the prevention of fungal HAIs increasingly important. However, in immunocompromised patients, such as Hematopoietic Stem Cell Transplantation (HSCT) recipients, Invasive Aspergillosis (IA) remains the most important cause of infection-related mortality [4]. In a large prospective registry of 234 HSCT recipients with invasive fungal infection, aspergillosis accounted for 59% of all invasive fungal infections and was associated with a 6-week mortality rate of 22% [5].

The role of ventilation systems (natural and mechanical) in the transmission of airborne infectious agents in buildings, particularly in medical facilities, lacks conclusive evidence. However, previous studies demonstrated the links between ventilation and spread of bioaerosols that include fungal propagules. Most studies in the country are generally focused on bacterial and viral sources of nosocomial infections and research on fungal species is limited. Consequently, this public tertiary hospital located in Manila lacks data on the existing fungi for both their non-air-conditioned and air-conditioned wards.

Hence, this study aimed to 1) isolate and identify existing fungal species in the health institution; 2) determine the environmental conditions and physical factors such as air temperature and relative humidity in the proliferation of fungi, and 3) determine the type of ventilation with the highest fungal load. Moreover, this study utilized a Six- Stage Andersen Air Sampler that made use of Malt Extract Agar with Chloramphenicol, incubated *in situ* for counting and identification to ensure isolation of human pathogenic molds of environmental origin. It will serve as a pilot and base line data for an environmental air sampling using Andersen Air Sampler in a medical institution for the whole country. Lastly, the study may serve as a guide for the hospital Infection Control Committee in developing protocols and proper management of indoor air quality and sanitation in the medical facility.

Methodology

Ethical Considerations

The study was submitted to and approved by the Research Ethics Board of the University of the Philippines Manila (UPM-REB). A permit for the conduct of the research was also secured from the Philippine General Hospital Expanded Hospital Research Office (PGH-EHRO). During the air sampling procedure, the researcher was accompanied by the site investigator in entering the ward. The trolley carrying the Andersen Air Sampler was then positioned in the center of each ward. The researcher also informed the ward occupants near the location of the air sampler instrument on the objectives and duration of the study conducted.

Study Setting

The indoor air sampling was conducted at a public tertiary hospital in Metro Manila, Philippines, particularly on three of its 30 air-conditioned wards and another three of its 16 non-air-conditioned wards. The three wards under the Department of Surgery (Ward 2-Male Surgery, Ward 4-Female Surgery, and Ward 6-Pediatrics Surgery) were the sampling sites for the non-air-conditioned wards (NACWs). These wards house patients who have undergone invasive

Table 1. *Room dimensions of the six selected sampling sites*

Ward	Room Dimensions (m)
Non-Air-conditioned Wards	
Ward 2- Department of Surgery (Male) Ward 4- Department of Surgery (Female) Ward 6- Department of Surgery (Pediatrics)	27 x 13 27 x 13 27 x 13
Air-conditioned Wards	
Room 434 Room 435 Room 436	6 x 9 6 x 9 6 x 9

medical procedures, predisposing these patients to opportunistic infections. On the other hand, ACW 1 (Room 434), ACW 2 (Room 435), and ACW 3 (Room 436) located at the 4th floor of the central block building were the sampling sites for the air-conditioned wards (ACWs). The main lobby at the ground floor and the nurse station at the fourth level of the hospital served as the control areas of the study for the NACWs and ACWs respectively. The control sites were selected due to their proximity to the sampling sites and similarity of environmental conditions and physical factors. All of the air-conditioning units in ACWs do not have High-Efficiency Particulate Air (HEPA) filters. The presence of construction or renovation site within the 500 meters radius of the sampling site was set as the exclusion criteria of the study. Table 1 indicates the room dimension of the six selected sampling sites.

Study Population, Sample Size, and Sampling Technique

The selected air-conditioned and non-air-conditioned wards of the hospital served as the study population of this research. The study followed a purposive sampling design wherein the sampling sites were equal to six wards only and two control areas based on the capacity of the researcher to analyze the number of isolates. Three from 30 air conditioned wards and another three from 16 non air conditioned wards in the hospital were selected.

Preparation of Malt Extract Agar with Chloramphenicol

For the fungal inoculation through Andersen air impaction method, the culture medium used was Malt Extract Agar (MEA) supplemented with chloramphenicol (20 g Malt Extract, 15 g Agar, 0.200g chloramphenicol, 1 L deionized water) from Gregory Gilbert Laboratory (2000). A 27- ml MEA was poured in 90-mm Andersen plates. In one sampling process, six MEA agar plates were used yielding 12 plates including the duplicate. Consequently, a total of 96 MEA plates were produced for the eight sampling sites.

Determination of Environmental Conditions and Physical Parameters

In coordination with the Philippine Astronomical, Geophysical and Astronomical Services Association (PAG-ASA), the environmental factors and physical parameters including temperature and relative humidity in the sampling sites during the date of sampling were obtained. The

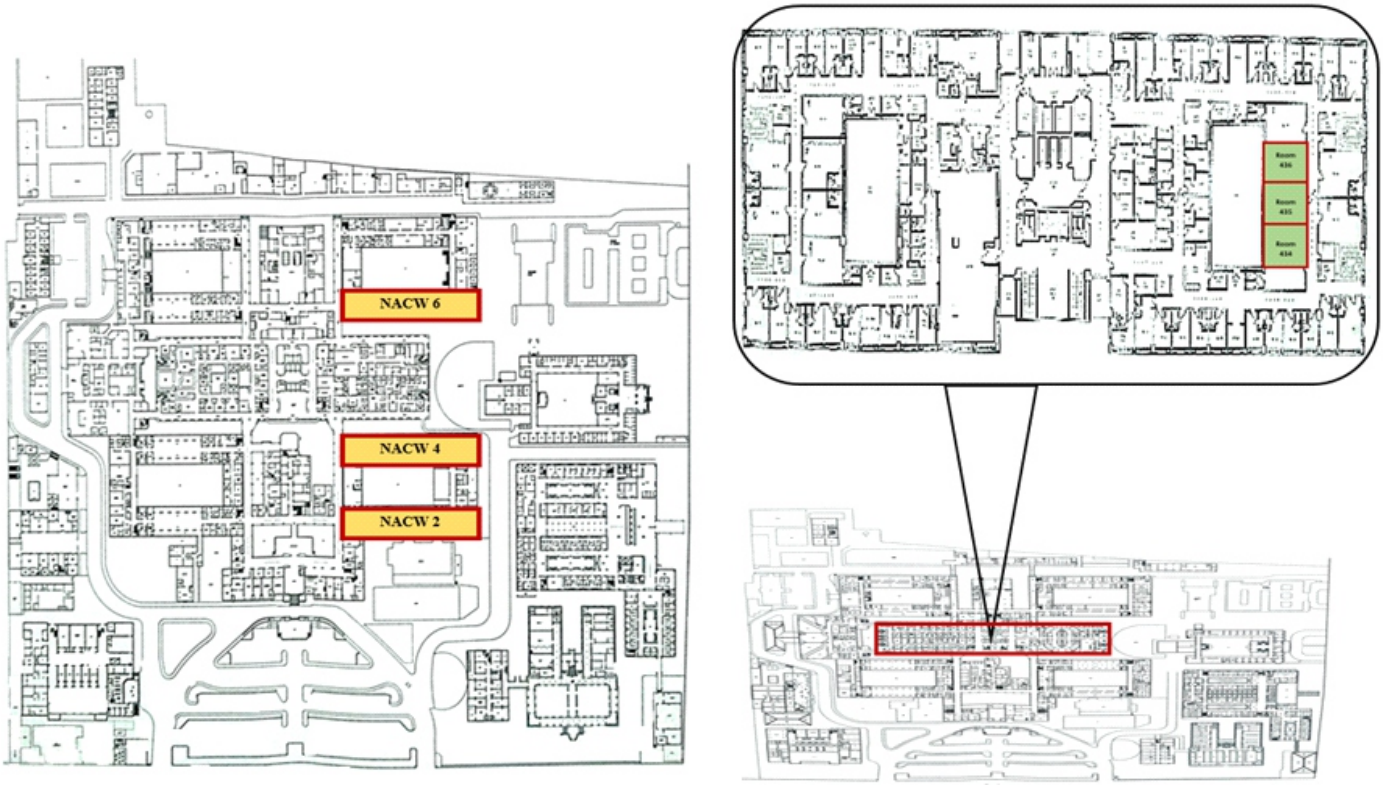

Figure 1. *Map of six selected sampling sites for NACWs and ACWs*
Source: Philippine General Hospital Office of Engineering and Technical Services

temperature and relative humidity inside the NACWs and ACWs were measured using a calibrated digital thermometer and hygrometer (HT-96A), respectively.

Indoor Air Sampling using Andersen Air Sampler

Indoor air sampling was conducted on April 16, 2018 at three ACWs, three NACWs, 4th Level Nurse Station and Main Lobby of a public tertiary hospital in Metro Manila. According to the Sanitary Department of the hospital, wards were cleaned early morning (7:00 a.m.) and late in the afternoon (2:00 p.m.). Air sampling was conducted from 10:00 a.m. to 1:45 p.m. before the second scheduled time of cleaning.

The use of Six Stage Andersen Air Sampler (Thermo Scientific N6 Cascade Impactor) borrowed from the UP Manila College of Public Health (Department of Environmental and Occupational Health) followed the NIOSH protocol (Method 0800 – NMAM, 1998). The air sampler was placed on top of a medical trolley to have an altitude of one meter above the floor and for easy mobility. During the air sampling procedure, the researcher was accompanied by the site investigator in entering the ward. The trolley carrying the Andersen Air Sampler was then positioned at the center of each ward. The sampling time was five minutes at 28.3 L/min. and done twice. The estimated duration of sampling per site was 15 minutes inclusive of the decontamination of the instrument and surface of the medical trolley with 70% isopropyl alcohol followed by the replacement of MEA plates for the second round of sampling. The decontamination of the sampler and the replacement of seeded plates were done on top of the medical trolley which also carried the two storage boxes.

Surface Sampling on Air Conditioning Units

The air conditioning units in the three ACWs were tested for fungi through surface sampling via swab method. The sterile swab was lightly rolled three times back and forth on the filter of the unit. The area for swabbing was guided with 1.5 by 1.5 inches template. The cotton swab applicator was then streaked on MEA plates supplemented with Chloramphenicol and incubated at 37 °C for five days. Swabbing was done in duplicate for all the air conditioning units in the three ACWs.

Incubation of Inoculated Malt Extract Agar with Chloramphenicol

After the sampling procedure, the inoculated MEA plates with Chloramphenicol were incubated at 37° C in the incubator (Walk-in Lab line S.N 147) of the Department of Medical Microbiology (DMM) Laboratory. The culture medium used was not selective and the overgrowth of microorganisms was inevitable. Thus, the analyses of the fungal colonies were conducted for two days (third and fifth day) during the five day incubation period. The number of Colony Forming Units (CFUs) was determined per cubic meter of air (CFU/m^3) [6].

Morphological Characterization of Fungal Isolates

On the fifth day of incubation, the colonies considered morpho-culturally distinct or showing typical characteristics of suspected fungi were isolated and compared with each other. These isolates were inoculated or sub-cultured in MEA to ensure purity. Lactophenol cotton blue-stained Scotch preparations were done for the microscopic analyses [7]. The morphological characteristics of the vegetative mycelium and the reproductive structures were considered in the identification of the isolates. The identification of the fungal isolates was up to the species level for *Aspergillus* and genus level for other fungal isolates based on the Laboratory Identification of Emerging Pathogenic Molds [8]. The preliminary identification of the fungal isolates was verified by Ms. Mary Ann C. Sison, University Researcher II in the DMM-College of Public Health, University of the Philippines Manila. Three samples for each of the identified strains were maintained and preserved in MEA slants with mineral oil and stored inside a secured cabinet inside the DMM laboratory.

Statistical Analysis

The study utilized the Independent Sample t-Test to compare the means of the seven isolated fungal organism per ward to the corresponding mean values of the positive control sites: Main Lobby and Nurse Station for NACWs and ACWs respectively. The same statistical analysis was used to compare the average means of the NACWs (Ward 2-Male Surgery, Ward 4-Female Surgery, Ward 6-Pediatrics) and ACWs (Room 434, Room 435, Room 436) to the control sites. The analyses were run using the IBM SPSS Software 2018.

Results

The air sampling conducted among the three NACWs (Ward 2-Male Surgery, Ward 4-Female Surgery, Ward 6-Pediatrics), three ACWs (Room 434, Room 435, Room 436) and two comparison sites (Main Lobby and 4th Level Nurse Station) collectively yielded seven fungal strains which were

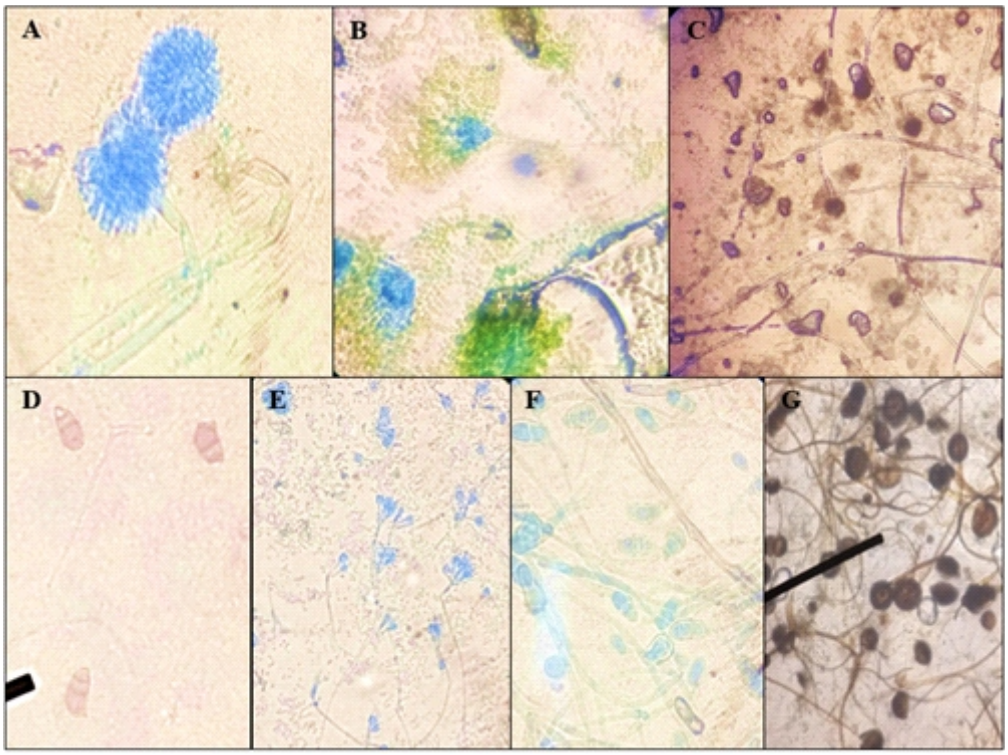

Figure 2. *The seven fungal strains isolated under the microscope (400x)* ***A)*** *A. fumigatus* ***B)*** *A. flavus* ***C)*** *A. niger* ***D)*** *Curvularia sp.* ***E)*** *Penicillium sp.* ***F)*** *Alternaria sp.* ***G)*** *Rhizopus sp.*

identified through morpho-cultural analysis. These are the following: *Aspergillus fumigatus, A. flavus, A. niger, Curvularia* sp., *Penicillium* sp., *Alternaria* sp. and *Rhizopus* sp.. These fungi have the ability to infect and proliferate inside a host with a compromised immune system. In using Andersen Air Sampler, particle size discrimination was possible as the air velocity increases through the smaller holes, thus, the species of fungi that can be impacted to each stage of the sampler can be assumed. The first stage that allows impaction of 6.8 microns and above the fungal spores was dominated with *Aspergillus niger* having a conidial size of 3- 6.5 µm followed by *A. fumigatus* (2-5.5 µm), and *Alternaria* sp. (7-17 µm). On the other hand, in Stage 2 of the sampler, the three *Aspergillus* species (*A. fumigatus, A. flavus* and *A. niger*) have the most number of fungal colonies, because of the size discrimination capacity of the equipment that allows the lodgement of 2.5-5.5 µm conidial size to 4.7-7 µm size range of perforations in this stage. Moreover, the third stage of the sampler was occupied mostly by the three *Aspergillus* (*A. fumigatus, A. flavus* and *A. niger*). Moving on to the sixth stage of the equipment, the fungal species with a larger conidial size (*Curvularia* sp., *Alternaria* sp. and *Rhizopus* sp.) were not apparent on this stage, however, the *Aspergillus* species were still observed due to their very variable conidial size range [9].

The environmental sampling using Andersen Air Sampler in Ward 2 revealed that the mean CFU/m³ of *A. fumigatus* and *A. niger* was 96.55% and 71.56% higher in this NACW as compared to the Main Lobby (control site). The differences in CFU/m³ between the Main Lobby and Ward 2 for *A. fumigatus* ($0.018 < 0.05$) and *A. niger* ($0.045 < 0.05$) were both significant. The mean CFUs of *A. flavus, Curvularia* sp., *Penicillium* sp., *Alternaria* sp., and *Rhizopus* sp. in Ward 2 showed lesser mean values when compared to the Main Lobby. The five mentioned organisms also displayed no significant differences between the two sampling sites.

On the other hand, *A. flavus* has a higher mean CFU/m³ in Ward 4 than in the Main Lobby of the hospital which also displayed a significant difference with a p-value of $0.009 < 0.05$. In addition, the mean CFU/m³ of *A. flavus* in Ward 4 was 123.80% higher when compared with mean values in the Main Lobby. The fungi *A. fumigatus, A. niger* (with highest number of isolated colonies), and *Penicillium* sp. also revealed a higher mean CFU/m³ in Ward 4 than in the Main Lobby, however, these three organisms showed no significant differences when compared to the control site. This is due to the minimal differences in the mean CFU/m³ of the two sampling sites. The mean CFU/m³ of organisms *Curvularia* sp., *Alternaria* sp., and *Rhizopus* sp. at the Main Lobby showed higher values than in Ward 4, however, no

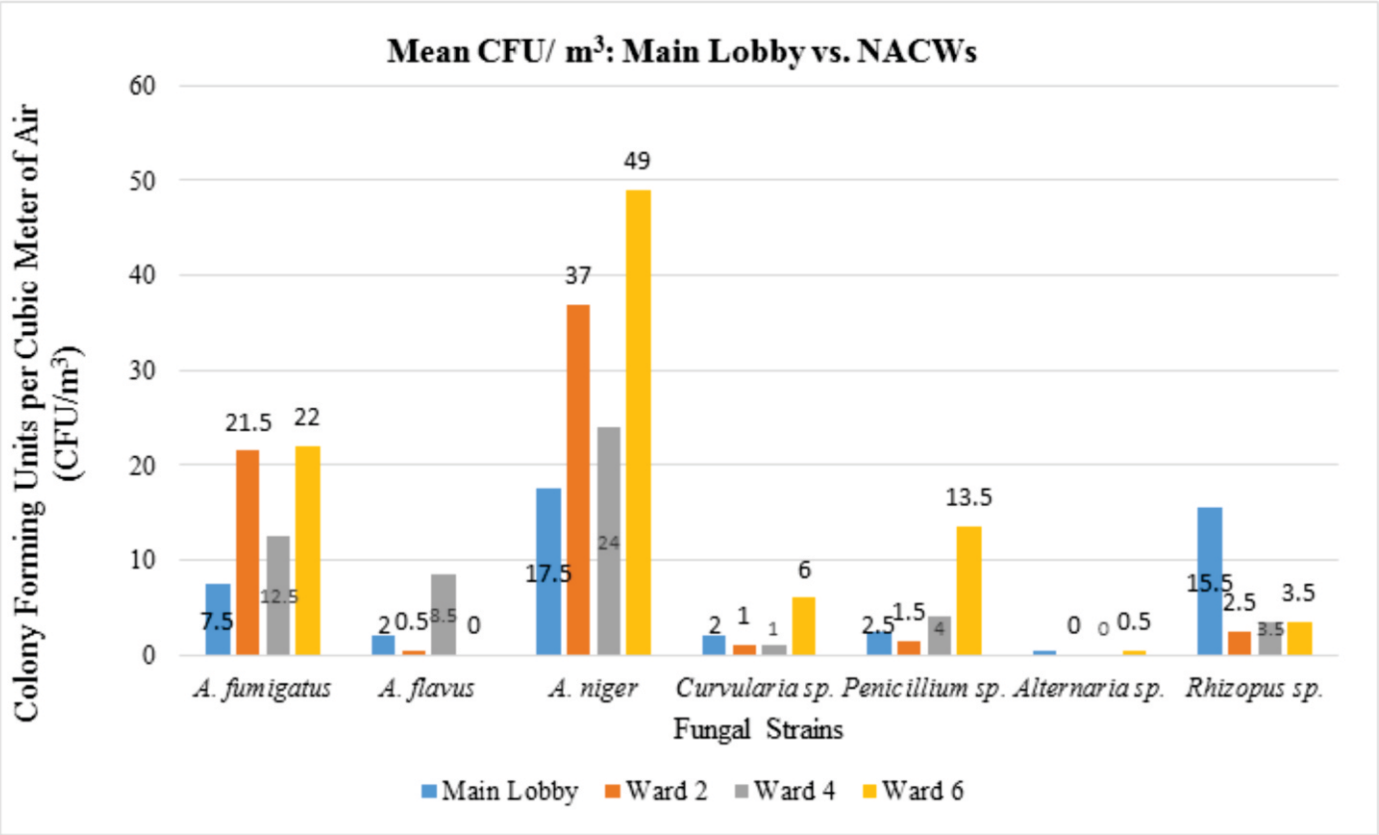

Figure 3. *Mean CFU/m³ values of Main Lobby and NACWs (Ward 2, Ward 4 and Ward 6)*

significant difference in CFU/m³ was observed between the two sampling sites.

For the third sampling site under the NACW, Ward 6 displayed a higher mean CFU/m³ for *A. fumigatus* (98.30%), *A. niger* (94.74%), and *Penicillium* sp. (137.5%) as compared with the values in the Main Lobby. The differences in CFU/m³ between the Main Lobby and Ward 6 for *A. fumigatus* (0.027 < 0.05), *A. niger* (0.048 < 0.05) and *Penicillium* sp. (0.020 < 0.05) were all significant. The fungi *Curvularia* sp., *Aspergillus flavus,* and *Rhizopus* sp. had a higher mean CFU/m³ in Ward 6 than in Main Lobby, however, the differences in values were not significant. Lastly, the mean CFU/m³ in *Alternaria* sp. for both Ward 6 and the control site was of the same value at 0.5.

Overall, the average mean of *A. fumigatus* (82.35%) and *A. niger* (72.33%) was higher among NACWs than the control site (Main Lobby) and showed significant differences for both organisms. Apparently, the CFU/m³ of *A. flavus*, *Curvularia* sp., *Penicillium* sp. and *Rhizopus* sp. from NACW were all higher when compared with the Main Lobby, however, the differences between the two values showed no significance.

The first sampling site under the ACWs, Room 434, when compared to the comparison site (Nurse Station) revealed a higher *A. flavus* (123.80%) and *A. niger* (186.44%) mean CFU/m³ in the former sampling site. The differences in CFU/m³ between the Nurse Station and Room 434 for *A. flavus* (0.005 < 0.05) and *A. niger* (0.002 < 0.05) were both significant. The ACW Room 434 gave a higher mean CFU/m³ for *Penicillium* sp. and *Alternaria* sp. than the Nurse Station. However, no significant differences among the values were observed due to the minimal discrepancy between the two mean values. This was also seen among the isolated organisms, *A. fumigatus*, *Curvularia* sp., and *Rhizopus* sp., but in contrast, the mean CFU/m³ of these two organisms was higher in the Nurse Station than inside Room 434. As seen in Figure 4, the dominant fungus found in the indoor sample (*A. flavus*) was different from that in the outdoor sample. Moreover, there was a big discrepancy between the values of *A. flavus* in Room 434, an implication that the fungus originated from the inside environment and 521 further investigation of the sources of contamination must be warranted.

A. fumigatus (180.57%) and *A. niger* (184.31%) had a higher mean CFU/m³ in Room 435 than in the Nurse Station.

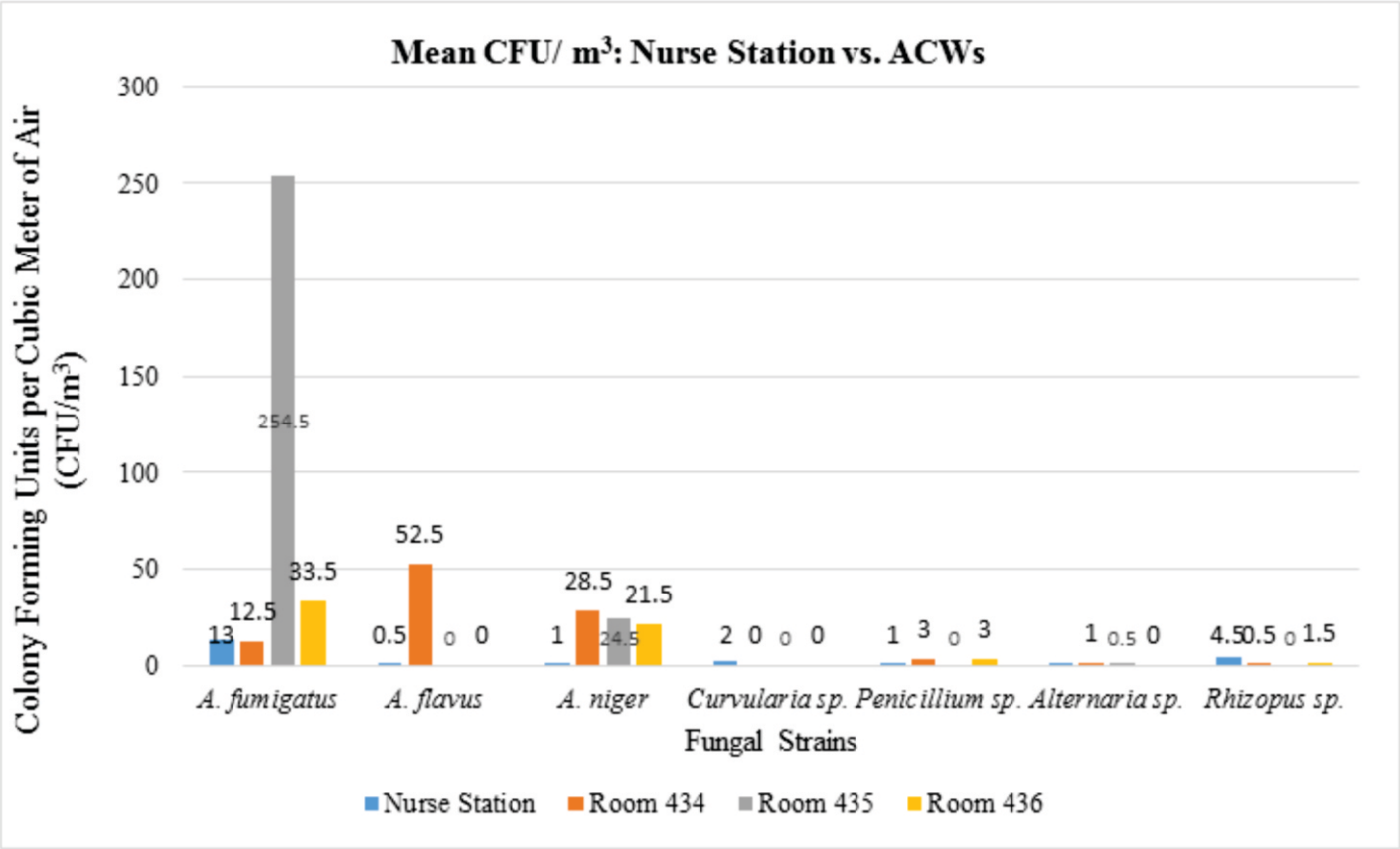

Figure 4. *Mean CFU/m³ values of Nurse Station and ACWs (Room 434, Room 435 and Room 436)*

The differences in CFU/m³ between the Nurse Station and Room 434 for *A. fumigatus* (0.009 < 0.05) and *A. niger* (0.006 < 0.05) were both significant. On the other hand, *A. flavus*, *Curvularia* sp., *Penicillium* sp., and *Rhizopus* sp. had a higher mean CFU/m³ in the Nurse Station than in Room 435, but displayed no significant differences when the values of each fungal organisms were compared. Moreover, *Alternaria* sp. showed the same mean value for both Room 435 and Nurse Station.

The last sampling site under the ACW, Room 436, displayed a higher mean CFU/m³ for *A. fumigatus* (with the highest number of isolated colonies), and *A. niger.* These two fungal organisms showed significant differences in the mean CFU/m³ when compared with the mean values in the Nurse Station with the p-values of 0.024 < 0.05 and 0.017 < 0.05, respectively. Moreover, the mean CFU/m³ in Room 436 for *A. fumigatus* was 88.17% higher than the mean values in the control site and 182.22% for *A. niger*. On the other hand, *A. flavus, Curvularia* sp., *Alternaria* sp., and *Rhizopus* sp. also showed a higher mean CFU/m³ in Room 436 than in the Nurse Station but the statistical test revealed no significant differences among the two sampling sites for the four isolates. The *Penicillium* sp. isolate had a higher mean CFU/m³ in Room 436 than in the control site but showed no significant difference.

Overall, the average mean value of *A. fumigatus* among all ACWs was 87.16% higher than in Nurse Station, 196.22% higher for *A. flavus* and 184.51% higher for *A. niger*. While *Penicillium* sp. mean average of CFU/m³ for ACWs also showed a higher value, its difference when compared to the control site was insignificant. For *Curvularia* sp. and *Rhizopus* sp., the mean CFU/m³ of the Nurse Station was higher than that of the ACWs, however, the discrepancies between the two compared values were also insignificant. The ACWs were not contaminated with *Curvularia* sp., which was isolated in the Nurse Station. The fungal isolate, *Alternaria* sp., showed no difference in value for the Nurse Station and ACWs.

Fungi were also isolated from the filters of the three air conditioning units of the ACWs through swabbing method and yielded the following fungi: *A. fumigatus, A. flavus, A. niger*, and *Penicillium* sp. Table 2 summarizes the mean CFU/m³ of the four isolated fungi in the air conditioning units for each of the ACWs.

Table 2. *Mean CFU/m³ of the four isolated organisms in the filters of air conditioning units for each of the ACWs.*

ACWs	*A. fumigatus*			*A. flavus*			*A. niger*			*Penicillium sp.*		
	CFUs/m³	Air Sampling	Swab Method	CFUs/m³	Air Sampling	Swab Method	CFUs/m³	Air Sampling	Swab Method	CFUs/m³	Air Sampling	Swab Method
Room 434	22	✓	✓	32	✓	✓	15	✓	✓	2	✓	✓
Room 435	55	✓	✓	2	✗	✓	7	✓	✓	0	✗	✗
Room 436	4	✓	✓	0	✗	✗	10	✓	✓	2	✓	✓

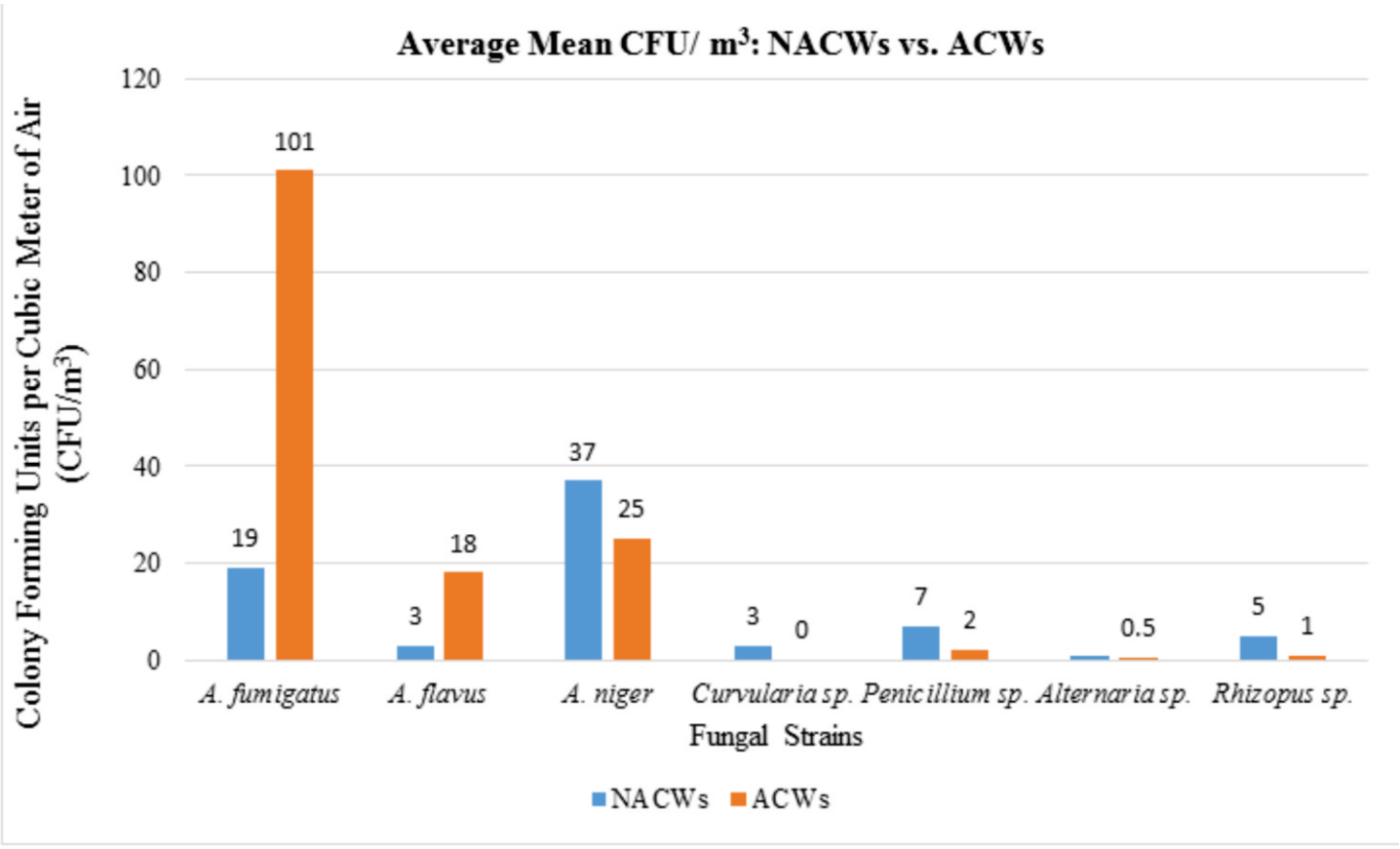

Figure 5. *Average Mean CFU/m³ values of NACWs (Ward 2, Ward 4, Ward 6) and ACWs (Room 434, Room 435, Room 436)*

Lastly, the average CFU/m³ of all NACWs and ACWs was compared. As shown in Figure 5, *A. fumigatus* and *A. flavus* had a higher mean CFU/m³ in ACWs than in NACWs. Both fungi had significant differences when the two groups of sampling sites were compared with a p-value of 0.048 < 0.05 and 0.038 < 0.05, respectively. In addition, the mean CFU/m³ of *A. fumigatus* was 136.66% higher in ACWs than in NACWs while ACWs was also 142.85% higher in *A. flavus* than the other group of sampling sites. The fungi *A. niger, Curvularia* sp., *Penicillium* sp., *Alternaria* sp., and *Rhizopus* sp. had a higher mean CFU/m³ in NACWs than in ACWs, however, there were very minimal differences in values between the two values for each of the organisms thus resulting to non-significance of the differences when treated with statistical analysis.

This study measured the environmental temperature of each of the sampling sites. As shown in Table 3, NACWs with an apparent higher environmental temperature had a lower average number of fungal isolates (72.66 CFU/m³) when compared to ACWs having a mean total of 150.66 CFU/m³. Another established physical factor affecting fungal growth was relative humidity. Table 4 indicates the relative humidity of each of the sampling and the total number of fungal isolates. The NACWs with a lower relative humidity yielded a lower number of fungal isolates when compared

Table 3. *Temperature of the sampling sites and the total mean number of fungal isolates obtained*

Non-Air-conditioned Wards (NACWs)			Air-conditioned Wards (ACWs)		
Sampling Site	Temperature	Total Number of Fungal Isolates	Sampling Site	Temperature	Total Number of Fungal Isolates
Ward 2	35.9 °C	65	Room 434	27.8 °C	100
Ward 4	33.95 °C	57	Room 435	26.05 °C	281
Ward 6	32.35 °C	96	Room 436	27 °C	71
Average Number of Fungal Isolates		72.66	Average Number of Fungal Isolates		72.66
Control Sites					
Sampling Site		Temperature			Total Number of Fungal Isolates
Main Lobby		33.25 °C			95
4th Level Nurse Station		32.55 °C			45

Table 4. *Relative humidity of the sampling sites and the total mean number of fungal isolates obtained*

Non-Air-conditioned Wards (NACWs)			Air-conditioned Wards (ACWs)		
Sampling Site	Relative Humidity	Total Number of Fungal Isolates	Sampling Site	Relative Humidity	Total Number of Fungal Isolates
Ward 2	47.5%	65	Room 434	54.5%	100
Ward 4	48.5%	57	Room 435	55.5%	281
Ward 6	44%	96	Room 436	58%	71
Average Number of Fungal Isolates		72.66	Average Number of Fungal Isolates		150.66
Control Sites					
Sampling Site		Relative Humidity			Total Number of Fungal Isolates
Main Lobby		53.5%			95
4th Level Nurse Station		56.5%			45

to ACWs with a higher relative humidity. Lastly, the varying dimension of the wards was also considered a physical factor affecting the number of fungal organisms isolated. In this study, NACWs had a larger area (27 x 13 m) than the ACWs (6 x 9 m) and there were lower fungal isolates in NACWs than in ACWs.

Discussion

The hospital as a health care facility has also become a source of infection with the presence of different microbiological agents, including viruses, bacteria, and fungi. The acquisition of these infections in a medical institution is termed as Health Care Associated Infections (HAIs). The sampling conducted among the eight sampling sites using Andersen Air Sampler has collectively isolated seven fungal organisms. The following fungi were identified through morphological-microscopic analysis: *A. fumigatus, A. flavus, A. niger, Curvularia* sp., *Penicillium* sp., *Alternaria* sp. and *Rhizopus* sp. These organisms were mesophilic in nature, however they also possess varying tolerances and in this study, they were able to grow at 37°C.

The most dominant fungal specie among the NACWs was *A.niger* followed by two other *Aspergillus* species (*A. fumigatus* and *A. flavus*). On the other hand, *A. fumigatus* was the most observed isolate among ACWs followed by the two organisms of the same genus. Members of the genus *Aspergillus* possess the ability to grow in a high osmotic pressure, *Aspergillus* species are highly aerobic and found in almost all oxygen-rich environments where they commonly grow as molds on the surface of a substrate as a result of the high oxygen tension [10]. Moreover, the low molecular weight of *Aspergillus conidia* was one factor for the abundance of these species in the air and had an important influence in their pathogenicity [11]. The small conidial size of these organisms allows them to stay suspended in the air as compared to other fungi with larger spores such as (*Curvularia* sp. and *Alternaria* sp.) that take lesser time to settle. In addition, *Aspergillus* species with a spore size of 2.5-5.5 microns has a settling velocity of 0.095 feet per minute and its settling rate is faster (0.59 feet per minute) in fungal organisms with a higher diameter [12]. The most dominating species for both NACWs and ACWs belong to the same genus *Aspergillus*. This only suggests the abundance and ability of this genus.

Evidently, the ACWs showed a higher number of fungal isolates. In particular, *A. fumigatus* and *A. flavus* colonies in ACWs were higher than in NACWs. The ubiquitous nature of the *Aspergillus* species and slow settling rate due to small spore size made it the most dominant fungal organism retrieved in the air sampling conducted.

The difference between the hospital Main Lobby and the NACWs was significant for *A. fumigatus* and *A. niger* in which the mean CFU/m^3 of the two organisms was higher in NACWs. This may imply that the environment in the NACWs was conducive for the fungal growth and proliferation of the organisms. The difference in the altitude of the Main Lobby and the NACWs was also a contributing factor for the result. The NACWs in the second floor were more exposed to upward circulating wind current that carries fungal spores. The air inside these wards was more saturated with fungal load than the outdoor air. Even with a higher environmental temperature and lower relative humidity than the Main Lobby, the NACWs' air sampling still showed more fungal isolates. In addition to this, the Main Lobby was also in proximity to the hospital atrium where a small garden was located. However, the chance that the results of the study will be treated as incidental findings cannot be ruled out due to the limitation of the study design (Descriptive, Cross-sectional) since this type of study design only gives a snap shot of the population and the air sampling was conducted only once.

Lastly, when the average mean CFUs of the ACWs was compared with the mean value in the Nurse Station, *A. fumigatus, A. flavus*, and *A. niger* showed significant differences. Accordingly, there was a higher fungal load inside the ACWs than the comparison site (Nurse Station). This may suggest that these rooms allowed fungal growth. The swabbing of air conditioning units revealed similarities in the isolated organisms for both the culture in swab method and air sampling which may imply that the air conditioning unit allowed the proliferation of these organisms and as one probable niche of fungal air contaminants. The air conditioning unit served as a condensation source and held moisture that was vital for fungal growth. Moreover, low temperature and high relative humidity inside the rooms were possible physical factors that contributed to a high number of isolated fungal colonies. However, as mentioned earlier, the chance that the results of ACWs vs. Nurse Station will be treated as incidental findings cannot be ruled out due to the limitation of the study design (Descriptive, Cross- sectional) since this type of study design only gives a snap shot of the population and the air sampling was conducted only once.

The NACWs with an apparent higher environmental temperature had a lower average number of fungal isolates (72.66 CFU/m^3) when compared to ACWs having a mean total of 150.66 CFU/m^3. According to Morris *et al.* (2000), high temperature and lack of water lower the probability of fungal growth [13]. Moreover, in congruence with the study of Kanamori *et al*. (2005), which characterized environmental fungal isolates in 10 hospital areas, the results showed that non-air-conditioned facilities in the hospital have a 62% lower fungal load than those areas equipped with air conditioning units [14]. On the other hand, NACWs with a lower relative humidity yielded a lower number of fungal isolates when compared to ACWs with a higher relative humidity. If the temperature increases, however, the amount of water vapor the air can hold increases, so the relative humidity decreases and vice versa. Most molds grow when the relative humidity of the air is 50-70% or higher [15]. Low moisture in the air that is necessary for fungal growth affected the low recovery of fungal organisms in non-air conditioned areas. The size of the sampling site affects the fungal load since a larger area provides more diluted air than a smaller area due to limited airflow [16]. This was also seen in the study of Gorny and Dutkiewicz (2002), with 10 non-air-conditioned hospital wards that were divided in two groups according to dimension. The first group of five had a larger area (20 x 16 m) and the second had 16 x 12 meters [17]. Their results showed that air sampling conducted in smaller wards yielded 45% more fungal isolates than the other group.

Conclusion

There were no strict numerical guidelines available for spore counts which were appropriate for assessing whether the contamination in a particular location is acceptable or not but the following threshold levels have been recorded for *Aspergillus* species: a) outdoor air = 5-10 CFU/m^3 b) HEPA- filtered air = <1 CFU/m^3 c) ward area with no air filtration = <5 CFU/m^3 [18]. The values of CFU/m^3 of most of the isolates not only by *Aspergillus* species showed non-compliance with the threshold level documented. This may only imply that the contamination for all of the sampling sites was not acceptable and poses an increased risk to susceptible individuals. For related research work and based on the results of this study, the following recommendations are made by the researcher:

1. Concerning the Hospital Infection Control Group:

1.1. Strict compliance with the standard operating procedures (SOPs) and guidelines of the National Standards in Infection Control for Healthcare Facilities by National Center for Health Facility Development, Department of Health and Philippine Hospital Infection Control Society (PHICS), particularly on dust elimination procedures;

1.2. Mechanical ventilations (e.g electric fan) brought by the patients inside the wards must also be checked for cleanliness.

1.3. Microbial air sampling for monitoring of air quality in the environment. Affected clinical areas should be monitored for ingress of dust in spite of preventive measures.

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