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Airborne Bacteria and Fungi Distribution Characteristics in Natural Ventilation System of a University Hospital in Thailand

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Abstract

The airborne bacteria and fungi distribution characteristics and their relationship with environmental factors in the natural ventilation system were investigated in a university hospital, Thailand. The 672 samples were collected in the outpatient and inpatient departments (OPDs and IPDs). The levels of carbon dioxide (CO_2), temperature, relative humidity, and the number of people were also recorded during sampling. The characteristics, size distribution, and concentration of bioaerosols were not affected by the tropical humid seasons but its levels were dependent on some environmental factors. The indoor and outdoor concentration (I/O) ratio and multiple regression analysis indicated the level of indoor airborne bacteria and fungi were affected by outdoor origins but mainly contributed by population occupied and humidity inside. There is no difference of dominant genera cultured from dry and wet season samples. However the respiratory factions of these bioaerosols were more than 60% when most of them

detected with particle size range of 2.1-3.3 μm. The results of this study can provide fundamental information about indoor air quality improvement and management in university hospitals that are located in the humid tropical zone of Thailand.

Keywords: Indoor air quality; Bioaerosol; Size distribution; Natural ventilation; Hospital

1. Introduction

The cross-contamination of airborne microbes suspended in the indoor air of hospitals affects the patients, medical staff, and all occupants. It is a cause of respiratory tract diseases and health risks such as nosocomial infection, asthma, tuberculosis, and sick building syndrome [Knutsen et al., 2012]. Previous studies indicated that the nosocomial infection rate in university hospitals in Thailand shows a higher rate (7.3-7.6%) than in other hospital types (4.9-6.0%) [Danchaivijitr et al., 2007]. Therefore, an indoor air quality management program has been strictly operated and monitored to reduce the public health risk.

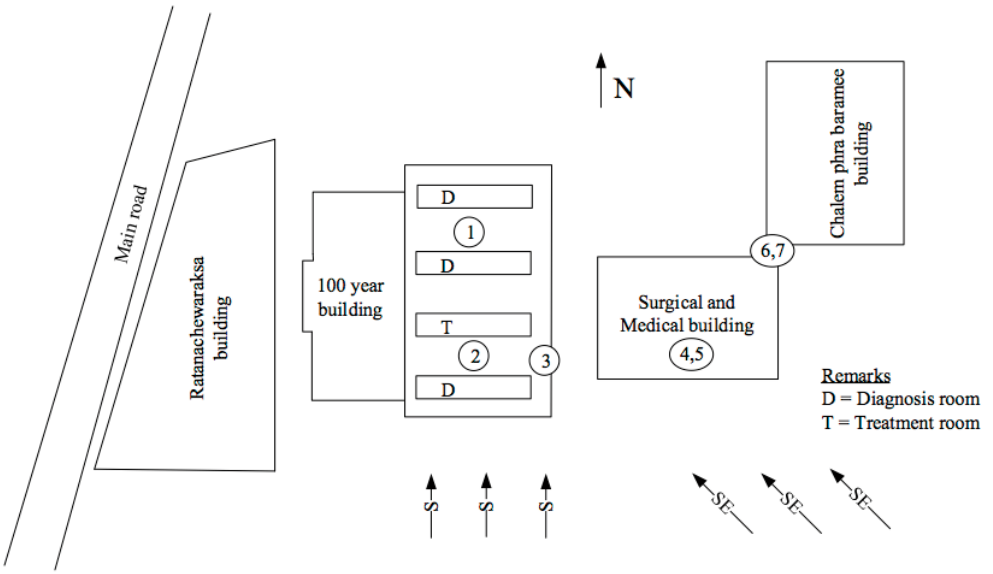
A majority of hospitals in Thailand use a natural ventilation system to reduce air pollutants and prevent the accumulation of bioaerosols in the buildings. Indeed, a high rate of natural ventilation can reduce airborne cross-infection despite the low operating cost. However, its effectiveness can be affected by environmental factors, such as temperature, relative humidity, and its seasonal variations. Moreover, the CO_2 level is related to the bioaerosols concentration in the buildings [Chao et al., 2002]. The relative humidity, generation source, and the genus of the microorganisms were related to the aerodynamic diameters of airborne bacteria and fungi. The small particles are able to travel into the pulmonary part of the lungs that affect human health. Therefore it is important to plan the improvement of indoor air quality programs in hospital. Especially in large-sized hospitals it is necessary to have effective indoor air quality management in case it provides a medium for airborne particles including infectious agents which can spread rapidly.

The aim of this study is to investigate the bioaerosol distribution characteristics during the wet and dry seasons, and the relationship between environmental factors and the bioaerosol levels in the natural ventilation systems of a university hospital. The results obtained may be basic useful information for further remediation and preventive design.

2. Materials and Methods

2.1 Site Description

The study site was a university hospital located in humid tropical climate of Southern Thailand. It has approximately 850 beds and over 2,000 staff employees, and provides primary to tertiary care treatments. The annual average temperature was 27.6 ^{o}C (25.9 - 30.0 ^{o}C). The seasons were classified by the amount of precipitation to be dry (February - mid-May) and wet seasons (mid-May - January).



No. 1 = M-OPD; No. 2 = E-OPD; No. 3 = Outdoor reference for OPD; No. 4 = M-IPD; No. 5 = S-IPD; No. 6 = Outdoor reference for M-IPD; No. 7 = Outdoor reference for S-IPD; S = Wind direction from South at OPDs; SE = Wind direction from Southwest at IPDs.

Figure 1. Sampling locations at the M-OPD, E-OPD, M-IPD and S-IPD.

2.2 Sampling Site

The service areas investigated included the Medical Outpatient Department (M-OPD), the Eye Outpatient Department (E-OPD), the Medical Inpatient Department (M-IPD) and Surgical Inpatient Department (S-IPD). All OPDs are located on the first floor of the main building with an opening space next to the corridor (Figure 1) where the area is approximately 430 m^2. The IPDs are located on the 5^{th} and 9^{th} floors of a 13-floor building that is about 700 m^2. There are ceiling fans installed on the ceiling between the patient beds and the nurse station. The tilted windows are open all the time and protected by mosquito screens. Air samples were collected from all working sites in the middle of the room. The three outdoor air samples were also collected.

2.3 Sampling Time

The air sampling was carried out from February to April (dry season) and from October to December (wet season). These periods of time comprised the peak duration with low precipitation, relative humidity, and the highest temperature in the dry season, and the peak duration with high precipitation, relative humidity, and the lowest temperature in the wet season (Figure 2).

Each sampling site included four points at indoor and three points at outdoor sites. It was performed 8 times once every two weeks for six months including duplicate samples. Two sets of samples, the morning (9:00-12:00) and the afternoon (13:00-16:00) were collected on each visiting day (weekday).

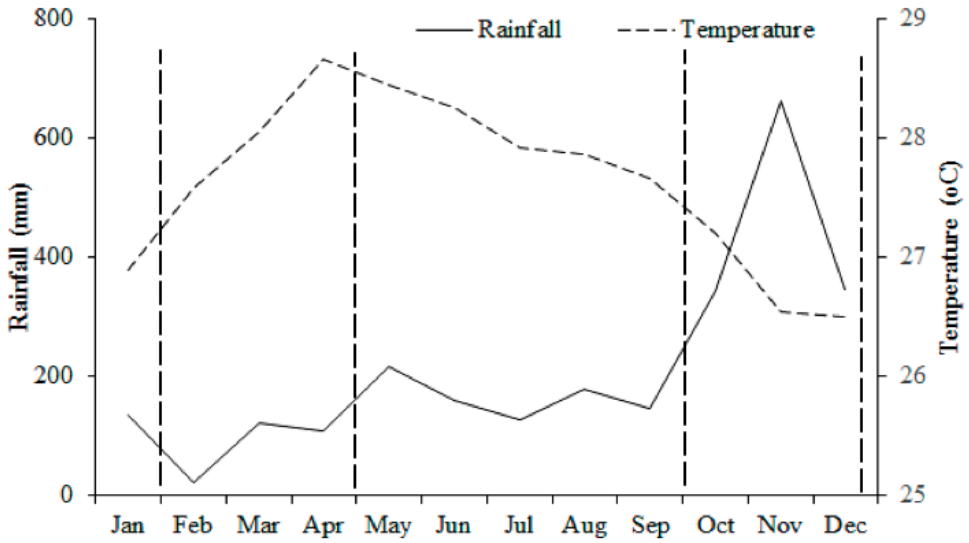


Figure 2. The sampling time of airborne bacteria and fungi.

Each set of samples at each sampling site contained 6 plates for bacteria and 6 plates for fungi analysis and it was done in duplicate. All sites were visited on the same day. The total sample was 672 samples (4,032 plates) included 384 samples (2,304 plates) from indoor and 288 samples (1,728 plates) from outdoor sites.

2.4 Airborne Bacteria and Fungi Measurement

The distribution of the particle sizes was determined using a six-stage viable cascade impactor (Model 10-800, Andersen Inc, USA) in which the aerodynamic diameter has six ranges: stage 1 (>7 μm), stage 2 (4.7-7.0 μm), stage 3 (3.3-4.7μm), stage 4 (2.1-3.3μm), stage 5 (1.1-2.1μm), and stage 6 (0.65-1.1μm). Before sampling, it was sterilized with 70% alcohol. The air samples were collected at 1.5 m above the floor (respirable zone) with an adjusted flow rate of 28.3 L/min for 5 minutes. The flow rate was calibrated at intervals and kept constant during each sampling. A trypticase soy agar medium was used for bacteria cultures and incubated at 37 °C for 1-2 days. A malt extract agar medium was used for fungi cultures and incubated at 25 °C for 3-5 days.

2.5 Identification of Airborne Bacteria and Fungi

The pure cultures of airborne bacteria were classified by Bergey's manual [Buchanan and Gibbons, 1974] whereas the classifications by fungi morphology according to the St-Germain and Summerbell (2011) method. The DNA extraction of cultures were slightly modified from the boiled-cell method previously described by Keegan et al. (2005).

The bacterial isolates were identified using PCR technique to amplify the complete 16S rRNA while the genera of fungal isolates was identified by PCR amplification of the 26S rRNA. DNA amplification of bacteria was performed by initial denaturation at 94 °C for

5 minutes, 30 cycles at 94 °C for 30 second (60 sec for fungi), annealing at 55 °C for 30 second (52 °C for 60 second for fungi), extension 72 °C for 30 second (120 second for fungi) and final extension at 72 °C for 5 minutes.

The amplicon was checked in 1% agarose gel by DNA electrophoresis. The UFUL-f and 802-r primers were used for DNA sequences for bacteria while F63-f primer for fungi. The DNA template 3-10 ng was mixed with 8.0 µl BigDye v3.1, 3.2 pmol primer and added the deionized water to a volume of 20 µl.

The 16rRNA sequences were as follows: an initial denaturation at 95 °C for 5 min, 30 cycles at 95 °C for 30 second (60 second for fungi), annealing 50 °C for 10 second (30 second for fungi), extension 60 °C for 4 minute and final extension at 60 °C for 4 minute.

The sequences of the PCR products and strain identifications were achieved using the nucleotide-nucleotide BLAST methods in the database of the National Centre for Biotechnology Information (NCBI) (http://ncbi.nlm.nih.gov).

2.6 Environmental Factor Measurements

The environmental parameters including temperature, relative humidity, room air velocity and CO_2 were collected during air samplings at the same sampling height. The temperature, relative humidity and room air velocity were measured using a VelociCalc® Air Velocity Meter (TSI Alnor, Model 9555, USA). The level of CO_2 was monitored by Gasmet™ (Gasmet Technologies, Model DX4000, Finland). The number of patients, medical staff employees, and all occupants were also counted during the sampling time.

2.7 Data Analysis

The Wilcoxon sign-ranked test was used to test the statistical differences of all measurement data between the dry and wet seasons. The Mann Whitney U-test was used to compare the bioaerosols concentration between indoor and outdoor and the OPDs and IPDs. Multiple regressions were used to test the relationships between bacteria and fungi quantities and the environmental factors.

3. Results

3.1 Concentration Distribution of Airborne Bacteria and Fungi

Table 1 showed the airborne fungi concentration in the dry season was similar to the wet season ($p>0.05$) either in indoor or outdoor samples and there was no difference between OPDs and OPDs. It is similar to the result of airborne bacteria measurement in OPDs. However the indoor and outdoor airborne bacteria concentration of the dry season at IPD sites were significantly higher than those of the wet season ($p <0.05$).

Moreover, the respiratory fractions (RFs) of airborne bacteria and fungi in the OPDs and IPDs showed similar values and were not significantly different between seasons ($p>0.05$). The respirable fractions of bacteria and fungi were 60.34 – 63.18% and 84.15 – 86.68% respectively. The high RF means high risk of infection in these people. However, the RFs of bacteria and fungi in this study were not different between the dry and wet seasons. This was due to the humid tropical environment that had only slightly different weather conditions in the area studied.

Table 1. Bioaerosols contamination and environment parameters in various locations of a university hospital.

	OPDs		p-value	IPDs		p-value
	Dry	**Wet**		**Dry**	**Wet**	
Bacteria						
Total(cfu/m^3)	853±407	826±410	0.645	289±160	154±60	0.000*
I/Oa	2.13	2.36		1.87	2.52	
Resp.b (cfu/m^3)	515±250	508±268		188±107	94±42	
RFc (%)	60.34	61.46	0.916	63.18	61.28	0.925
Fungi						
Total(cfu/m^3)	681±310	747±310	0.181	620±270	739±325	0.123
I/O	1.17	1.23		1.17	1.16	
Resp.a (cfu/m^3)	573±277	630±250		538±243	629±284	
RFb (%)	84.15	84.28	0.523	86.68	85.12	0.395
Occupancy level (Persons/m^2)	0.34±0.29	0.44±0.35	0.000*	0.095±0.017	0.100±0.017	0.007*
CO_2 (ppm)	495.99±31.31	509.94±44.62	0.000*	470.96±26.40	451.06±18.76	0.000*
Temperature (°C)	29.57±0.85	28.81±0.89	0.000*	29.60±1.09	29.19±1.30	0.142
Relative humidity (%)	68.77±4.19	73.48±3.78	0.000*	67.26±6.66	71.72±7.13	0.005*
Air velocity (m/s)	0.28±0.06	0.29±0.07	0.800	0.18±0.05	0.16±0.05	0.266
Outdoor bacteria	400±146	351±199	0.356	159±84	61±34	0.000*
Outdoor fungi	582±276	608±233	0.961	532±246	638±265	0.277

a Resp. - respirable concentration or sum of bioaerosols quantity measured at stages 3-6.

b RF - respirable fraction, percentage of respirable concentration/total concentration

* Significant at $p<0.05$

Table 2. Multiple regression models of total indoor airborne bacteria and fungi.

	β coefficient	SE	P-value	
Airborne bacteria				R=0.884
Constant	-6.109	1.433	0.000*	
Number of people	0.167	0.060	0.006*	
CO_2	2.814	0.569	0.000*	
Air velocity	0.432	0.104	0.000*	
Outdoor bacteria	0.495	0.049	0.000*	
Airborne fungi				R=0.722
Constant	-1.384	0.398	0.001*	
Humidity	1.544	0.228	0.000*	
Outdoor fungi	0.488	0.054	0.000*	

*= Significant at $p<0.05$

3.2 Relationship between Bioaerosols and Environmental Factors

Multiple regression models to predict indoor concentration of airborne fungi and bacteria showed high predictability, 0.884 and 0.722 for bacteria and fungi respectively. The results concerning the outdoor bacteria, CO_2, room air velocity, and number of people, all had an impact on indoor bacteria levels (Table 2). Meanwhile, outdoor fungi and relative humidity levels are correlated with indoor fungi concentrations.

3.3 Seasonal Distribution of Airborne Bacteria and Fungi Types

The predominant genera of indoor airborne bacteria identified in both seasons were *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., and *Corynebacterium* spp. For outdoor airborne bacteria, the main types were *Micrococcus* spp., *Staphylococcus* spp., *Bacillus* spp. and *Corynebacterium* spp. However the same predominant airborne fungi were found in both seasons from indoor and outdoor air sampling: i.e., *Cladosporium* spp., followed by *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp (Table 3).

3.4 Seasonal Size Distribution of Viable Bacteria and Fungi

The average size distribution of airborne bacteria and fungi in the indoor air showed similar patterns in both seasons (Figure 3). The size distribution of airborne bacteria during occupancy was the highest in stage 4 (2.1-3.3 μm) and stage 1 (>7 μm) while airborne fungi were at their highest at stage 4 (2.1-3.3μm).

Figure 4 presented a similar pattern of predominant genera of indoor and outdoor airborne bacteria and fungi in both seasons. The size distribution of *Staphylococcus* spp. and *Bacillus* spp. was detected mainly at >7μm, and *Micrococcus* spp. at 1.1-3.3 μm. The airborne fungi showed that *Cladosporium* spp. and *Penicillium* spp. had the highest size distribution at 2.1-3.3 μm and >7 μm for *Fusarium* spp. *Aspergillus* spp. were the highest at 2.1-4.7 μm.

Table 3. Airborne bacteria from various sites in the dry and wet seasons.

cfu/m^3	OPDs		O-OPD		IPDs		O-IPDs	
	Dry	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**
Bacteria								
Staphylococcus spp.	926	1,258	198	141	389	268	170	63
	59.47	60.31	40.08	35.79	55.10	57.63	34.91	31.98
Micrococcus spp.	361	495	205	155	184	113	212	92
	23.19	23.73	41.50	39.34	26.06	24.30	43.53	46.70
Corynebacterium spp.	149	198	42	49	56	35	42	7
	9.57	9.49	8.50	12.44	7.93	7.53	8.62	3.55
Bacillus spp.	121	135	49	49	77	49	63	35
	7.77	6.47	9.92	12.44	10.91	10.54	12.94	17.77
Total	1,557	2,086	494	394	706	465	487	197
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Fungi								
Cladosporium spp.	601	551	311	170	1,484	360	693	594
	54.10	50.64	59.35	46.32	67.30	41.47	57.99	56.04
Penicillium spp.	261	311	92	127	417	332	212	233
	23.49	28.58	17.56	34.60	18.91	38.25	17.74	21.98
Aspergillus spp.	121	128	50	35	191	113	120	113
	10.89	11.76	9.54	9.54	8.66	13.02	10.04	10.66
A. fumigatus	7	7	7	0	0	0	7	7
	0.63	0.64	1.34	0	0	0	0.59	0.66
Fusarium spp.	121	91	64	35	113	63	163	113
	10.89	8.36	12.21	9.54	5.12	7.26	13.64	10.66
Total	1,111	1,088	524	367	2,205	868	1,195	1,060
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

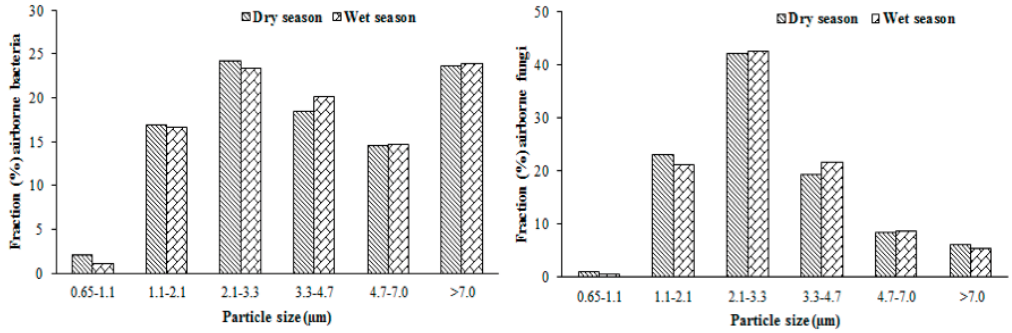


Figure 3. Size distribution of airborne bacteria and fungi.

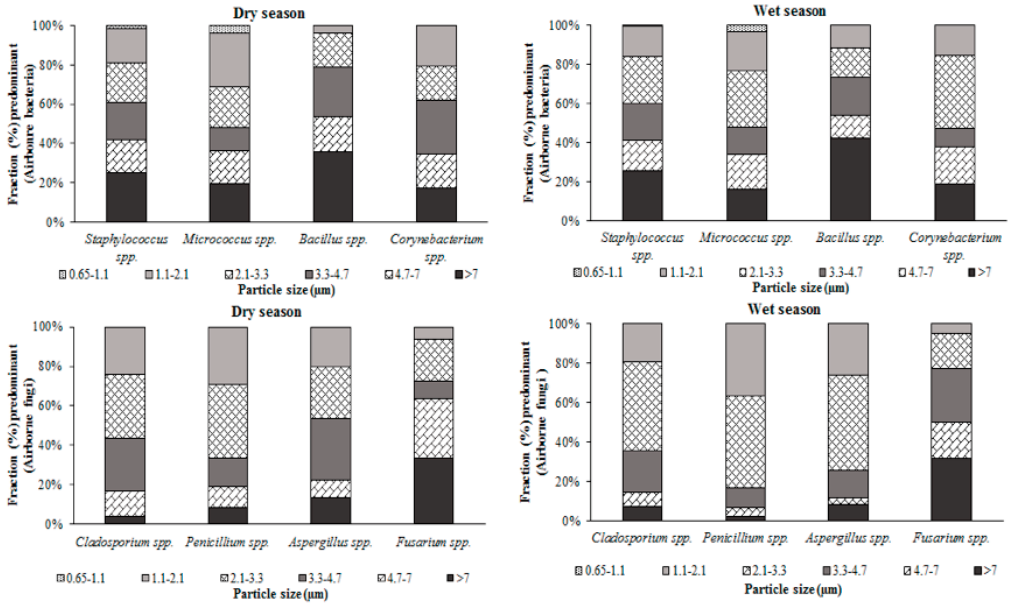


Figure 4. Size distribution of predominant airborne bacteria and fungi.

4. Discussion

To describe the concentration and distribution characteristics, the I/O ratio accompanied with other factors and result of multiple regression analysis in Table 2 were considered. Accordingly, various studies have been mentioned the penetration of outdoor generated particulate into indoor micro-environments has been found to play an important role [Diapouli et al., 2011; Huang et al, 2007]. If I/O ratio is near 1.0, it can predict that the concentration inside is directly relate to outdoor concentration and the ambient air penetration into the indoor environment which was found in the case of indoor area with open line. Meanwhile the I/O would be decreased when indoor area is enveloped [Cyrys et al., 2004]. In this study, the I/O ratio values at all sites investigated exceeded 1.0, this could be implied that the contamination from indoor origins was the main source of indoor microorganisms exists in all sites investigated.

The high indoor airborne bacteria depended on occupants level which was confirmed by the multiple regression analysis (Table 2) and was in accordance with other study [Hospodsky et al., 2012]. For airborne fungi contamination in the indoor area investigated, it was contributed from humidity and outdoor fungi. The I/O ratio exceed 1.0 was also described by the contamination contributed from indoor humidity source including rest rooms that are placed closed to patient bed in IPDs and waiting area of OPDs, wall made from plywood, and plumbing. From these results, the study about the relationship of its I/O ratio variation and indoor air quality levels may be useful for the development of an indoor air quality index in a public health work environment.

To describe the higher bacteria concentration existing in IPDs area during dry season, it depended on the activity and number of visitors. Table 1 and Table 2 showed the different occupant levels in IPD's rooms is the significant factor affected on airborne bacteria concentration. It was not similar to OPDs which located the center of the hospital located on the first floor and which have a high density of people throughout the year. Thus the indoor bacteria concentration and occupant level in this area were not different between seasons. The IPDs area are located on 5^{th} and 9^{th} floor of building and it has limit of visit time and has lower visiting people number than OPDs. Furthermore, the outdoor airborne bacteria concentration in wet season were lower concentration because the wet season in the study area has high rainfall and the rainfall increased the relative humidity level in the air, thus cleaning the air and decreasing the airborne bacteria level in this sites [Qian et al., 2012; Rangaswamy et al., 2012].

When a few meteorological factor ranges showed differences, these had no effect on fungi concentrations. It was different from other countries that locate in the region that the meteorological factors changes can be seen obviously [Ponce-Caballero et al., 2013].

The human activity, CO_2 concentration and occupant density can affect the airborne bacteria in the indoor air. Increases in the occupant density and CO_2 levels were significantly related to the bioaerosols accumulated in the building. These results were to those reported in previous studies (Fox et al., 2003; Mahyuddin et al., 2014; Meadow et al., 2014). CO_2 is relatively easy to be used as surrogate of indoor pollutants emitted by human when it correlates with human metabolism. Because both CO_2 and

bioeffluent generation rates depend on physical activity, the concentration of CO_2 and the odor intensity from human bioeffluents in a space exhibit a similar relationship with the number of occupants [Batog and Badura, 2013; Dougan and Damiano, 2014]. Thus there is suspicion for using to monitor indoor air quality and fresh air ventilation control relating population density [Yu et al., 2017].

A fungi concentration level in the indoor air was contributed by outdoor fungi. The outdoor fungi can flow pass through the opened window and door to inside of building that can increase indoor fungi level [Ponce-Caballero et al., 2013]. Furthermore, the high relative humidity of indoor air from human activity and building material can be potential cause of indoor fungi increase.

The airborne bacteria isolated may be different depending on the location of the sampling [Harper et al., 2013], but the main genera found in this study were mostly similar to those in other studies investigating indoor air in hospitals [Aboul-Nasr et al., 2011]. As described in the literature, the source of *Staphylococcus* spp. and *Micrococcus* spp. is mainly humans [Pastuszka et al., 2005]. In this study, the fraction of *Staphylococcus* spp. was obviously increased in indoor air and significantly related with occupant density which confirmed its source from human (Table 3).

The data showed that indoor concentrations of *Cladosporium* spp. increased in the dry season while *Penicillium* spp. increased in the wet season. These phenomena can be found in other building types such as domestic residences [Ponce-Caballero et al., 2013]. All predominant genera can be isolated from the environment that confirmed they also came from an outdoor source. However, the concentration of the indoor levels was higher than the outdoor levels which demonstrated the contribution of indoor environment and the accumulation of bioaerosols with insufficient ventilation.

Presently, *Cladosporium* spp., *Penicillium* spp., and *Aspergillus* spp. are increasingly considered as health risks for haematology, cancer, and transplantation patients [Aboul-Nasr et al., 2011]. The frequency of airborne fungi exposure is a cause of adverse human health effects such as allergy, asthma, allergic rhinitis and sick building syndrome.

In addition, the % size fraction of predominant bacteria isolated was not different between the two seasons. This may be because most bacteria came from human sources such as human activity, speaking, coughing and breathing that can account for all particle sizes from the smallest to the largest (<0.8 to 125μm). This is why all size fractions also found the same genera. The free airborne bacteria were found in a range of 1 to 2 μm while the bacteria clusters were found between 3 to 7 μm [Ghosh et al., 2013].

The % fraction of predominant fungi mostly showed the particle sizes in the range of 2.1-3.3 μm that fitted with the spores of *Penicillium* spp. (2.1-3.4 μm) [Reponen et al., 1988] and *Cladosporium* spp. spores (2.0-4.0 μm). Moreover, the pathogenic *Aspergillus* spp. which has a spore size of about 2.5-3.0 μm in diameter [Samson and van Reenen-Hoekstra, 1988], can penetrate into the lungs and be highly hazardous for human health. In addition the *Fusarium* spp. also has a large size (>7 μm). This is caused by Fusarium morphology that can appear in the form of macroconidia with a large diameter [Khokhar et al., 2015].

5. Conclusions

The airborne bacteria were obviously found in higher concentrations at OPDs than that of IPDs while the airborne fungi were found at similar levels across all the working sites. The statistical analysis, I/O ratio, and genera fraction results revealed that the main source of airborne bacteria and fungi contamination in indoor air of hospital wards investigated were occupant number and humidity respectively. The outdoor bacteria and fungi also contributed the indoor contamination level that was confirmed with genera type distribution. The genera identified are often found in the indoor air of the hospital and they were not of different types between the dry and wet seasons.. The size fraction showed the airborne bacteria were in free and combined form with other particles whereas the indoor airborne fungi were the conidia and spores distributed form. This information may be useful for determining suitable disinfection light bulk. The indoor air quality management should also concern the use of exhaust fan in restroom, enhance regular cleaning, and ventilate the attic and crawl spaces to prevent moisture build-up.

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References

Aboul-Nasr MB, El-Zoohri ANAE, Amer EM. Indoor airborne mycobiota of intensive care units in Assiut University Hospitals. Journal of Environmental Studies 2011; 7: 61-66.

Batog P, Badura M. Dynamic of changes in carbon dioxide cconcentration in bedrooms. Procedia engineering 2013; 57: 175-182.

Buchanan RE, Gibbons NE. Bergey's manual of determinative bacteriology. 8th ed. Lippincott Williams & Wilkins, Baltimore, Maryland, USA. 1974.

Chao HJ, Schwartz J, Milton DK, Burge HA. Populations and determinants of airborne fungi in large office buildings. Environmental health perspectives 2002; 110(8): 777-82.

Cyrys J, Pitz M, Bischof W, Wichmann HE, Heinrich J. Relationship between indoor and outdoor levels of fine particle mass, particle number concentrations and black smoke under different ventilation conditions. Journal of Exposure Analysis and Environmental Epidemiology 2014; 14(4): 275-283.

Danchaivijitr S, Judaeng T, Sripalakij S, Naksawas K, Plipat T. Prevalence of nosocomial infection in Thailand 2006. Journal of the Medical Association of Thailand 2017; 90 (8): 1524-9.

Diapouli E , Eleftheriadis K, Karanasioul AA, Vratolis S, Hermansen O, Colbeck I, Lazaridis M. Indoor and Outdoor Particle Number and Mass Concentrations in Athens. Sources, Sinks and Variability of Aerosol Parameters. Aerosol and Air Quality Research 2011; 11: 632-642.

Dougan DS, Damiano L. CO2-based demand control ventilation-Do risks outweigh potential rewards?. ASHRAE journal 2014; October: 47-53.

Fox A, Harley W, Feigley C, Salzberg D, Sebastian A, Larsson L. Increased levels of bacterial markers and CO2 in occupied school rooms. Journal of Environmental Monitoring 2003; 5(2): 246-252.

Ghosh A, Chaudhary SA, Apurva SR, Tiwari T, Gupta S, Singh AK, et al. Whole-Genome Sequencing of Micrococcus luteus Strain Modasa, of Indian Origin. Genome announcements 2013; 1(2): e00076-13.

Harper TAM, Bridgewater S, Brown L, Pow-Brown P, Stewart-Johnson A, Adesiyun AA. Bioaerosol sampling for airborne bacteria in a small animal veterinary teaching hospital. Infection Ecology & Epidemiology 2013; 3(1): 1-7.

Huang H, Cao JJ, Lee SC, Zou CW, Chen XG, Fan SJ. Spatial Variation and Relationship of Indoor/Outdoor PM2.5 at Residential Homes in Guangzhou City, China. Aerosol Air Qual. Res 2007; 7(4): 518– 530.

Hospodsky D, Qian J, Nazaroff WW, Yamamoto N, Bibby K, Rismani-Yazdi H, et al. Human Occupancy as a Source of Indoor Airborne Bacteria. PLoS ONE 2012; 7(4) e34867: 1-10.

Keegan H, Boland C, Malkin A, Griffin M, Ryan F, Lambkin H. Comparison of DNA extraction from cervical cells collected in PreservCyt solution for the amplification of Chlamydia trachomatis. Cytopathology : official journal of the British Society for Clinical Cytology 2005; 16(2): 82-87.

Khokhar MK, Sharma SS, Hooda KS, Roat BL. Morphological and molecular characterization of Fusarium spp. causing post flowering stalk rot of maize. Vegetos- An International Journal of Plant Research 2015; 28(1): 113-121.

Knutsen AP, Bush RK, Demain JG, Denning DW, Dixit A, Fairs A, Greenberger PA, Kariuki B, Kita H, Kurup VP, Moss RB, Niven RM, Pashley CH, Slavin RG, Vijay HM, Wardlaw AJ. Fungi and allergic lower respiratory tract diseases. The Journal of allergy and clinical immunology 2012; 129(2): 280-291.

Mahyuddin N, Awbi HB, Alshitawi M. The spatial distribution of carbon dioxide in rooms with particular application to classrooms. Indoor and Built Environment 2014; 23(3): 433-448.

Meadow JF, Altrichter AE, Kembel SW, Kline J, Mhuireach G, Moriyama M, et al. Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. Indoor air 2014; 24(1): 41-48.

Pastuszka JS, Marchwinska-Wyrwal E, Wlazlo A. Bacterial aerosol in Silesian hospitals: Preliminary results. Polish Journal of Environmental Studies 2005; 14(6): 883-890.

Ponce-Caballero C, Gamboa-Marrufo M, Lopez-Pacheco M, Ceron-Palma I, Quintal-Franco C, Giacoman-Vallejos G, Loria-Arcila JH. Seasonal variation of airborne fungal propagules indoor and outdoor of domestic environments in Merida, Mexico. Atmosfera 2013; 26(3): 369-377.

Qian J, Hospodsky D, Yamamoto N, Nazaroff WW, Peccia J. Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom. Indoor air 2012; 22: 339-351.

Rangaswamy BE, Prakash KK, Fernandes F, Ravishanker BV, Shanmukhappa S. Occurrence of microbes causing respiratory ailments in a garden of Davangere. Indian Journal of Allergy, Asthma and Immunology 2012; 26: 61-65.

Reponen T, Grinshpun SA, Conwell KL, Wiest J, Anderson M. Aerodynamic versus physical size of spores: Measurement and implication for respiratory deposition. Grana 2001; 40: 119-125.

Samson RA, van Reenen-Hoekstra ES. Introduction to Foodborne Fungi. 3rd, editor. Centraal-bureau voor Schimmelcultures: Baarn, Delft, Germany. 1988.

St-Germain G, Summerbell R. Identifying fungi. Korea: Star publishing, Korea. 2011.

Yu S, Ma X, Yu Z, Zhang G, Feng G. The determination method of fresh air volume in buildings for different density of the crowd. Procedia Engineering 2017; 205: 2577-2584.