Original Article

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## Bioaerosols Assessment in the Intensive Care Units of a Tertiary Care Hospital.

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[3]งานส่งเสริมสุขภาพ โรงพยาบาลสงขลานครินทร์ [4]หน่วยวิจัยการจัดการสุขภาพและความปลอดภัยแบบองค์รวมในชุมชน คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อ.หาดใหญ่ จ.สงขลา 90110
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## บทคัดย่อ

**วัตถุประสงค์:** การศึกษาภาคตัดขวางนี้ มีวัตถุประสงค์เพื่อพรรณนาความเข้มข้นและขนาดของจุลชีพในหออภิบาลผู้ป่วยหนักอายุรกรรม (MICU) และศัลยกรรม (SICU) ของโรงพยาบาลสงขลานครินทร์ และปัจจัยที่มีความสัมพันธ์กับจุลชีพในอาคาร

**วัสดุและวิธีการ:** ขนาดและความเข้มข้นของจุลชีพในอากาศหออภิบาลผู้ป่วยหนักตรวจวัดด้วยเครื่องเก็บตัวอย่างจุลชีพในอากาศชนิด 6 ชั้น ระหว่างเดือนมิถุนายน พ.ศ. 2554-กุมภาพันธ์ พ.ศ. 2555 จากนั้นนำตัวอย่างที่ได้ วิเคราะห์หาชนิดของจุลชีพเด่น ด้วยวิธี PCR ปัจจัยด้านสิ่งแวดล้อมดำเนินการตรวจวัดควบคู่ไปกับการเก็บตัวอย่างจุลชีพโดยใช้เครื่องมือชนิดอ่านค่าโดยตรง

**ผลการศึกษา:** ปริมาณแบคทีเรียและราทั้งหมดภายใน MICU มีค่า 214.22±93.27 และ 194.25±74.83 cfu/$m^3$ ในขณะที่ SICU ระหว่างเปิดระบบฆ่าเชื้อโรคด้วยแสงอัลตราไวโอเลต (UVGI) มีค่า 274.44±140.75 และ 234.39±115.60 cfu/$m^3$ และปิดระบบ UVGI มีค่า 515.12±246.75 และ 531.41±337.65 cfu/$m^3$ ตามลำดับ ความเร็วลมภายนอกอาคารน้อยกว่า 1 เมตรต่อวินาที พัดผ่านอาคารก่อสร้าง ส่งผลให้มีการสะสมของปริมาณจุลชีพ เช่น *A. fumigatus* และ *A. flavus* บริเวณใกล้เคียงอาคารก่อสร้าง โดยชนิดของแบคทีเรียเด่น คือ *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp. ในขณะที่ราเด่นคือ *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp., และ *Fusarium* spp. การใช้ระบบ UVGI และความเร็วลมภายใน SICU มีผลกับปริมาณแบคทีเรียภายใน SICU ในขณะที่ปริมาณราภายใน SICU ขึ้นอยู่กับปริมาณราภายนอกอาคาร ความเร็วลม ความชื้นสัมพัทธ์ และอุณหภูมิภายใน SICU อย่างมีนัยสำคัญทางสถิติ แต่ไม่พบปัจจัยใดๆที่มีความสัมพันธ์กับปริมาณจุลชีพภายใน MICU

**สรุป:** เพื่อลดปริมาณจุลชีพในอาคาร ควรเพิ่มความเร็วลมภายในอาคาร และติดตั้งระบบ UVGI ใน ICU ที่มีพื้นจำกัด


**คำสำคัญ:** ก่อสร้าง, ขนาดอนุภาค, แบคทีเรียในอากาศ, ปัจจัยสิ่งแวดล้อม, ราในอากาศ


## Abstract:

**Objective:** This cross-sectional study describes the characteristics and size distributions of bioaerosols in the Medical Intensive Care Unit (MICU) and Surgical Intensive Care Unit (SICU) of Songklanagarind Hospital. The relationship of the investigated factors on indoor bioaerosol concentration was clarified.

**Material and Method:** A six-stage viable cascade impactor was used to assess the concentrations and size distributions of bioaerosols in the ICUs from June 2011 to February 2012. The predominant bioaerosols were further analyzed by the polymerase chain reaction (PCR) technique. The meteorology factors were simultaneously measured with the viable microbes.

**Results:** The total indoor bacteria and fungi concentrations at the MICU were 214.22±93.27 and 194.25±74.83 cfu/$m^3$ while at the SICU during on-ultraviolet germicidal irradiation (UVGI) system they were 274.44±140.75 and 234.39±115.60 cfu/$m^3$ and during the off-UVGI they were 515.12±246.75 and 531.41±337.65 cfu/$m^3$, respectively. Since air passed through the MICU at a velocity of less than 1 m/s from a nearby construction site, accumulations of outdoor bacteria and fungi such as *A. fumigatus* and *A. flavus* were sampled at the site. The predominant bacteria and fungi in ICUs were *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp. and *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp., and *Fusarium* spp., respectively.

The functioning of the UVGI and the room air velocity depended significantly on the indoor bacteria concentration in the SICU while the indoor fungi concentration depended significantly on the outdoor fungi concentration, room air velocity, indoor relative humidity and indoor temperature.

**Conclusion**: To decrease the indoor bioaerosol concentrations, the room air velocity should be increased and the UVGI system should be installed in the limited space of the ICUs.

**Keywords**: Airborne bacteria, Airborne fungi, construction work, environmental factor, size distribution

## Introduction

Microbiological contamination in terms of poor indoor air quality can lead to major respiratory system infections in immunocompromised patients[1,2] and sick building syndrome of hospital occupants.[3] Nosocomial infections in hospitals were determined to be a major contribution to the morbidity and mortality rates in patients[4,5] especially patients admitted at the Intensive Care Units (ICUs) which had the highest infection rates.[6] Hence, several methods to reduce the airborne microorganisms were implemented in the ICUs such as isolated and closed rooms and the admittance of the relatives of patients.[7] However, bioaerosols can be distributed from the peeling of skin, the gastrointestinal tract and the clothing of personnel.[8] The airborne bacteria which can affect humans are *Mycobacterium tuberculosis*, *Acinetobacter* spp., *Bacillus* spp., and *Staphylococcus aureus*, and the airborne fungi are *Aspergillus* spp., *Rhizopus* spp., *Acremonium* spp., *Fusarium* spp. and *Pseudallescheria boydii*.[9] Bioaerosols can be generated from several sources that include the inside and outside of the ICUs. The outdoor air may have an effect on the indoor air quality in ICUs via the moving in and out of people and the conductive air. In addition, many studies reported the effects of nearby activity on the air quality in a hospital, for example heavy truck traffic can increase the amount of particulate matter in the environment of a hospital.[10] Therefore, the air quality management outside an ICU is also important and should be a concern.

The ICUs in a tertiary care hospital should be influenced less by the outdoor environmental factors because ultraviolet germicidal irradiation (UVGI) systems and heat, ventilation and air-conditioning (HVAC) systems were installed in the ICUs to reduce viable *Mycobacterium tuberculosis* and other microorganisms.[11,12] However, if a good maintenance program of these systems is not in place, their performance will decrease over time. There are limited data on the indoor air quality management and environmental factors related to indoor bioaerosol concentrations in the ICUs in Thailand. Therefore, bioaerosol samples were taken in the ICUs of a tertiary care hospital to determine the efficiency of the maintenance program.

This study aimed to investigate the airborne microbiological characteristics in the ICUs of a tertiary care hospital. The concentration, size distribution, and types of airborne bacteria and fungi are presented. The relationships of outdoor air quality and some environmental factors both from inside and outside of the ICU which included occupant density,

frequency of room cleaning, UVGI operation, temperature, relative humidity, wind velocity and direction, and the distances from the construction sites were also determined. These results will be useful in planning and management of indoor air quality especially in the ICUs or other special wards in hospitals to prevent nosocomial infections of patients and also prevent sick building syndrome of health care workers in the tropical zone.

## Material and method

### Study area

This is a cross-sectional study that was performed in the ICUs of Songklanagarind Hospital, Prince of Songkla University, Thailand from June 2011 to February 2012. The bioaeroral samplings were taken during the rainy season (August to September 2011) and there was construction work near the study area. An aerial view of the bioaerosol sampling sites is shown in Figure 1. The Internal Medical Intensive Care Unit (MICU) (No. 2) and Surgical Intensive Care Unit (SICU) (No. 5) represent the indoor environments of the ICUs. The outdoor environment of the ICUs, represented by the open-air areas which were located away from the construction work site at 43 (No. 1, $O_c$), 81 (No. 3, $O_1$), and 143 meters (No. 4, $O_2$), were simultaneously collected on the same day as the indoor bioaerosal samples.

### Bioaerosol sampling and meteorology data

The six-stage viable Andersen cascade impactor (The Thermo Scientific®, USA) used in this study was designed and developed to measure the viable microbial load. The aerodynamic diameter

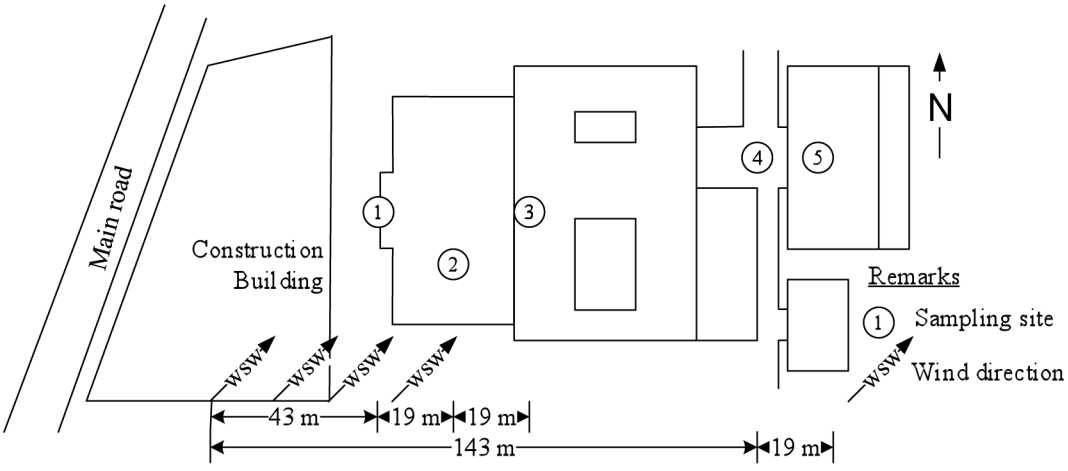


**Figure 1** Map of the construction work site and sampling locations.

Whereas: No.1 = nearby the construction work site ($O_c$); No. 2 = MICU; No. 3 = outdoor reference for MICU ($O_1$); No.4 = outdoor reference for SICU ($O_2$); No. 5 = SICU; WSW = west-southwest

ranges for each stage in the cascade impactor were: >7.0 μm (stage 1), 4.7-7.0 μm (stage 2), 3.3-4.7 μm (stage 3), 2.1-3.3 μm (stage 4), 1.1-2.1 μm (stage 5) and 0.65-1.1 μm (stage 6). To determine the bioaerosol size distribution and concentration with the Andersen cascade impactor, the air flow rate was set at 28.3 l/min (NIOSH Method 0801)[13] and the sampling time was set at 5 minutes in order to prevent overloading the plates (the pilot run time of bioaerosol samplings were taken at 5 and 10 minutes, respectively). All samples were taken at a height of 1.5 meters from the floor to represent the human breathing zone environment. At each sampling location site, the sampling period was between 09:00 and 12:00 to represent the morning period, and between 13:00 and 16:00 for the afternoon period. Each bacteria and fungi sample at the MICU was collected for 3 days X 4 times (2 in the morning and 2 in the afternoon) X duplicate samples whereas at the SICU during the on and off-UVGI periods samples were collected 3 days X 2 times (once in the morning and once in the afternoon) X duplicate samples. The meteorology factors such as temperature (°C), relative humidity (% RH) and wind velocity (meters per second, m/s) were measured simultaneously with bioaerosol sampling by direct-reading instruments (VelociCal, TSI, Germany). The outdoor wind velocity and direction data were from the Kohong Agrometeorological Station (Songkhla). The highest frequency of wind directions in each sampling location are represented in percentage (Table 1).

### Bioaerosol and polymerase chain reaction (PCR) analysis

A 70% ethanol solution was used to disinfect the instruments prior to air sample collection. Nutrient agar was used for bacteria cultures and potato dextrose agar was used for fungi cultures. The bacteria cultures were incubated at 37 °C for 2 days and the fungi cultures at 20 °C for 5 days by a trainee. The concentrations of microorganisms were expressed in colony forming units per cubic meter, $cfu/m^3$ following these converting equations.

The concentrations of microorganisms of each stage ($cfu/m^3$)

$$= \frac{\text{colony forming count at that stage (colony forming units, cfu) x } 10^{-3}}{\text{sampling flow rate (liter per minute, lpm) x sampling times (minute)}}$$

The total concentrations of microorganisms of each stage ($cfu/m^3$)

$$= \frac{\text{the summation of colony forming count at stage 1 to 6 (colony forming units, cfu) x } 10^{-3}}{\text{sampling flow rate (liter per minute, lpm) x sampling times (minute)}}$$

**Table 1** Indoor and outdoor general characteristics (mean±S.D.) of the ICUs and meteorology data at all sampling sites.

| Sampling site | Occupant density (people/m$^2$) | Cleaning (times/day) | UVGI system | Temperature (°C) | Relative humidity (%) | Air flow velocity (m/s) | Wind direction (%) | Total fungi | Total bacteria |
|---|---|---|---|---|---|---|---|---|---|
| MICU | 0.10±0.03 | 4 | No | 26.01±1.07 | 66.98±5.61 | 0.14±0.03 | - | 194.25±74.83 | 214.22±93.27 |
| Outdoor 1 ($O_1$) | - | - | No | 29.43±1.02 | 70.48±3.54 | 0.80±0.73 | WSW (50.00) | 688.60±80.40 | 384.28±170.82 |
| SICU | 0.39±0.05 | 3 | UVGI Off | 25.57±0.71 | 60.86±3.60 | 0.14±0.06 | - | 531.41±337.65 | 515.12±246.75 |
| | 0.34±0.09 | | UVGI On | 26.17±1.26 | 57.02±2.27 | 0.11±0.03 | - | 234.39±115.60 | 274.44±140.75 |
| Outdoor 2 ($O_2$) | - | - | No | 30.45±1.39 | 63.95±6.43 | 1.35±0.65 | ESE (33.20) | 585.39±532.77 | 96.58±38.49 |
| Nearby construction site ($O_3$) | - | - | No | 29.90±0.07 | 70.50±0.31 | 1.24±0.43 | WSW (50.00) | 2,065.37±234.20 | 455.83±134.65 |

**Remark**: WSW = West-southwest, ESE = East-southeast, % of wind direction represented the most direction on wind in that sampling day.
The occupant density = the number of people in each area/the room area (m$^2$)

Only airborne bacteria and fungi samples of the morning phase were uniformly dispersed in all of the six stages. They were purely cultured and classified by Bergey's manual[14] for bacteria. The fungi were classified by form, shape, spore color and color by the St-Germain and Summerbell method.[15] The purified samples were then sent to the Faculty of Science, Mahidol University for bio-aerosol species identification by the following method.

The deoxyribonucleic acid (DNA) extractions of bacteria and fungi used the modified boiled-cell method by Keegan et al.[16] The pellet was dissolved in 100-500 µl of TE Buffer (10 mM Tris-HCL pH 8.0 and 1 mM DTA), vortexed and held at 100 °C for 10-15 minutes. The suspension was centrifuged at 10,000-12,000 rpm for 5-10 minutes and 50-200 µl was kept in a freezer at 0-5 °C.

The molecular method was used to identify the airborne bacteria and fungi. The complete 16S rDNA gene (bacteria) was amplified by PCR using the primers UFUL-f: 5'-gCC TAA CAC ATg CAA gTC gA-3' and 802-r: 5'-TAC Cag ggT ATC TAA TCC-3'. While 26S rDNA gene (fungi) was amplified using the F63-f: 5'-GCA TAT CAA TAA GCG GAG GAA AAG-3' and LR-r: 5'-GGT CCG TGT TTC AAG ACG G -3'.

The reaction mixture consisted of 2-5 µl DNA template, 0.4 µM dNTP, 0.4 µM each primer, 1xPCR buffer (10 mMKCL, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCL, 2 mM $MgSO_4$, 0.1% Triton X-100), 2 mM $MgCL_2$, 1 U Taq DNA Polymerase (BioLab) and deionized water was added to a volume of 20 µl DNA amplification was performed by initial denaturation at 94 °C for 5 minutes, 30 cycles at 94 °C for 30 seconds (60 seconds for fungi), annealing at 55 °C for 30 seconds (52 °C

for 60 seconds for fungi), extension 72 °C for 30 seconds (120 seconds for fungi) and final extension at 72 °C for 5 minutes.

The PCR products were purified and checked by electrophoresis on 1.0% agarose gel electrophoresis. The DNA sequences of bacteria used the same primers for bacteria (UFUL-f and 802-r) and for fungi (F63-f). The reaction mixture was 8.0 µl BigDye v3.1, 3-10 ng DNA Template, 3.2 pmol primer and deionized water was added to a volume of 20 µl. The 16rRNA and 26 rRNA sequences were as follows: an initial denaturation at 95 °C for 5 minutes, 30 cycles at 95 °C for 30 second (60 seconds for fungi), annealing 50 °C for 10 seconds (30 seconds for fungi), extension 60 °C for 4 minutes and final extension at 60 °C for 4 minutes.

The 16 rRNA and 26 rRNA products were sequenced by automated sequence analyzer (3100-Avant genetic analyzer). Sequence associations were determined using the nucleotide-nucleotide BLAST, which has known bacteria and fungi listed in the official databases of the National Centre for Biotechnology Information (http://ncbi.nlm.nih.gov).

### Statistical analysis

The descriptive statistics, that included percentage, mean, and standard deviation, were used to explain bioaerosol concentrations, environmental parameters and the data of the other general characteristics. To compare the differences of bioaerosol concentrations during the morning and afternoon periods, the t-test and Wilcoxon rank sum test were used to compare the results of the normal and skewed distribution, respectively. The general linear model (GLM) was performed to investigate the association between all variables and indoor bioaerosol concentrations by the R program. A p-value of $<0.05$ was considered significant.

## Results

### General characteristics of ICUs

The main structure of the ICUs was a concrete block building. The MICU was a large area of approximately 360 $m^2$ with a capacity of 10 beds for medical treatment of severe illnesses. It was located on the fourth floor and was 62 meters from a nearby construction work site and a main traffic road. The MICU area was separated into 2 sections: an infection control area which employed a UVGI system and a non-infection control area. The SICU area consisted of only an infection control area. The SICU was smaller than the MICU. The area of the SICU was about 80 $m^2$ and it had 10 beds for the care of critically ill surgical patients. The location of the SICU was on the third floor and was 162 meters from the construction site (Figure 1). The UVGI system in the SICU operated only when infectious-disease patients were admitted into the ward. The HVAC systems of the MICU and SICU were the same with double doors to protect the areas from contamination from outdoor bioaerosols. The general characteristics of the two ICUs and the meteorology data of each sampling site are shown in Table 1.

All of the total bacteria and fungi concentrations, even if the UVGI system was on, were higher in the SICU than in the MICU. The occupant density of the SICU was higher than that of the MICU. When the UVGI system was on, the relative humidity was lower than when it was off. The highest concentrations of outdoor bacteria and fungi

were observed at $O_c$ and were lower at $O_1$ and at $O_2$, respectively.

**Size distribution of airborne bacteria and fungi in the ICUs**

The total fungi and bacteria concentrations at the MICU were 194.25±74.83 and 214.22±93.27 cfu/$m^3$, respectively. The total fungi and bacteria concentrations at the SICU were 234.39±115.60 and 274.44±140.75 cfu/$m^3$, respectively, when the UVGI was on and 531.41±337.65 and 515.12±246.75 cfu/$m^3$, respectively, when the UVGI was off (Table 1). There was no significant difference of bioaerosol concentrations between morning and afternoon periods except the bacteria concentrations at $O_1$ and the functioning of UVGI at the SICU (Table 2 and 3). The size distribution of the fungi (Table 2) in the MICU peaked at 1.1-2.1 μm while the size distribution of the fungi in the SICU peaked at 2.1-3.3 μm (UVGI system on and off). The indoor/outdoor ratio (I/O) in this study showed that most fungi in the ICUs were lower than the outdoor counterparts (I/O<1). The size distribution of bacteria (Table 3) in the MICU peaked at 2.1-3.3 μm. However, the size distributions of bacteria in the SICU during the off and on conditions of the UVGI system peaked at 1.1-2.1 μm. In the SICU, the I/O ratios of bacteria were higher (I/O>1) in both situations of UVGI system on or off. However, the I/O ratio of bacteria in the MICU was lower than 1.

**Factors related to indoor bioaerosol concentration**

The outdoor bioaerosol concentration (cfu/$m^3$), period of day (morning=1/afternoon=2), functioning of UVGI system (No=0/Yes=1), indoor air velocity (m/s), indoor RH (%), indoor temperature ($^oC$), outdoor air velocity (m/s), outdoor RH (%), outdoor temperature ($^oC$), and number of people in each period of day were used to carry out the factors that affected the indoor bioaerosol concentration (cfu/$m^3$). Finally two equations were used to predict the indoor bioaerosols in this study. The prediction calculation for the total bacteria concentration in the SICU was done based on significant parameters by the following equation:

(1) Y = 1114.93 - 304.40(UVGI, on, off) - 4556.22 (indoor air velocity, m/s) where Y = total bacteria (cfu/$m^3$).

However, there were no significant parameters to predict the total bacteria concentration in the MICU.

The prediction calculation for the total fungi concentration in the SICU employed the following equation:

(2) Y = -6501.84 + 0.43 (total outdoor fungi, cfu/$m^3$) - 5236.58 (indoor air velocity, m/s) + 75.61 (indoor relative humidity, %) + 110.71 (indoor temperature, $^oC$) where Y = (total fungi, cfu/$m^3$).

Likewise, there were no significant parameters to predict the total number of fungi concentration in the MICU.

The total concentration of indoor bacteria depended on UVGI usage (on or off). The bioaerosol concentrations increased when UVGI-off and decreased when the room air velocity increased, whereas the outdoor fungi concentration, indoor air velocity, relative humidity and temperature significantly influenced the indoor fungi concentration in the SICU.

**Table 2** Fungi concentrations (mean±S.D., cfu/m$^3$) in different size ranges at all sampling points.

| Stage No. (size range, μm ) | MICU (n=12; 12) | | $O_1$ (n=4; 4) | | SICU UVGI Off (n=6; 6) | | SICU UVGI On (n=6; 6) | | $O_2$ (n=4; 4) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Morning | Afternoon | Morning | Afternoon | Morning | Afternoon | Morning | Afternoon | Morning | Afternoon |
| 1 (>7) | 11.19±5.20 | 10.69±5.48 | 40.64±7.50 | 25.61±1.25 | 21.20±14.13 | 24.74±15.40 | 31.80±22.07 | 15.31±10.20 | 37.69±27.45 | 42.40±19.67 |
| 2 (4.7-7) | 12.37±5.81 | 17.08±11.12 | 37.10±22.49 | 21.79±14.16 | 42.40±15.46* | 32.78±10.03 | 23.56±22.72 | 15.31±10.20 | 32.98±26.76 | 54.18±38.92 |
| 3 (3.3-4.7) | 31.80±20.48 | 20.51±10.81 | 113.07±14.99 | 113.37±35.40 | 74.20±54.39* | 87.75±25.75 | 43.58±42.16 | 41.22±17.43 | 74.20±56.09 | 209.66±215.09 |
| 4 (2.1-3.3) | 66.54±49.29 | 62.23±40.78 | 337.46±52.47 | 326.86±112.44 | 213.19±202.69* | 153.12±56.35 | 67.14±42.99 | 81.27±15.40 | 148.41±159.17 | 259.13±276.44 |
| 5 (1.1-2.1) | 64.78±29.87 | 76.95±34.04 | 167.84±22.49 | 182.27±37.06 | 212.01±257.27** | 176.48±59.78 | 73.03±50.01 | 58.89±7.36 | 109.54±93.69 | 179.34±169.48 |
| 6 (0.65-1.1) | 6.48±6.86** | 7.85±8.28* | 3.53±4.99 | 7.66±5.83 | 12.96±8.16* | 11.79±7.46 | 8.24±4.08 | 9.42±2.04 | 9.42±2.04 | 14.13±10.60 |
| **Total** | **193.17±89.24** | **195.33±65.96** | **699.65±64.96** | **677.56±121.18** | **575.97±517.53*** | **487.85±105.94** | **247.35±174.87** | **221.47±48.23** | **412.45±337.68** | **758.54±711.09** |
| P-value | >0.05$^+$ | | >0.05$^+$ | | >0.05$^+$ | | >0.05$^{++}$ | | >0.05$^+$ | |

**Remark**: *means I/O>1 which the indoor air was contaminated with the microorganisms. **means I/O>1.5 which the indoor air was contaminated with the microorganisms

$^+$Wilcox rank sum test $^{++}$t-test

**Table 3** Bacteria concentrations (mean±S.D., cfu/m$^3$) in different size ranges at all sampling points.

| Stage No. (size range, μm ) | MICU (n=12; 12) | | $O_1$ (n=4; 4) | | SICU UVGI Off (n=6; 6) | | SICU UVGI On (n=6; 6) | | $O_2$ (n=4; 4) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Morning | Afternoon | Morning | Afternoon | Morning | Afternoon | Morning | Afternoon | Morning | Afternoon |
| 1 (>7) | 47.11±20.44 | 29.64±18.84* | 50.65±44.18 | 22.37±1.67 | 68.32±30.05** | 45.15±15.99** | 31.80±16.19** | 38.86±9.35** | 17.67±9.35 | 14.13±9.35 |
| 2 (4.7-7) | 34.16±21.97 | 22.48±6.37 | 42.40±40.75 | 25.91±3.33 | 88.34±76.69** | 51.63±35.53** | 38.87±12.74** | 25.91±7.36** | 10.60±6.12 | 10.60±9.35 |
| 3 (3.3-4.7) | 50.06±18.75 | 27.36±7.14* | 97.76±92.44 | 24.74±9.99 | 103.65±57.12** | 73.81±37.18** | 71.85±5.40** | 29.45±5.40* | 21.20±10.60 | 21.20±22.07 |
| 4 (2.1-3.3) | 55.36±22.86 | 42.70±29.70 | 85.98±74.48 | 58.01±27.90 | 128.39±56.13** | 123.09±64.00** | 94.23±73.30** | 36.51±20.09** | 24.74±10.60 | 20.02±15.93 |
| 5 (1.1-2.1) | 55.36±37.90* | 38.48±21.74 | 45.94±42.84 | 96.88±82.87 | 173.14±99.51** | 122.30±47.23** | 88.34±49.47** | 56.54±55.20** | 17.67±9.35 | 21.20±7.07 |
| 6 (0.65-1.1) | 14.13±8.94* | 11.58±10.03 | 12.96±12.41 | 37.10±52.47 | 30.62±13.38** | 21.79±4.45** | 21.20±10.60** | 15.31±7.36** | 9.42±5.40 | 4.71±2.04 |
| **Total** | 256.18±99.41 | 172.26±70.92 | 503.53±7.50 | 265.02±174.90 | 592.46±313.12** | 437.78±190.33** | 346.29±166.53** | 202.59±79.43** | 101.30±10.20 | 91.87±59.44 |
| P-value | >0.05$^+$ | | <0.05$^+$ | | >0.05$^{++}$ | | <0.05$^{++}$ | | >0.05$^{++}$ | |

**Remark**: *means I/O>1 which the indoor air was contaminated with the microorganisms. **means I/O>1.5 which the indoor air was contaminated with the microorganisms
$^+$Wilcox rank sum test $^{++}$t-test

### Size distributions of prevalent bioaerosols in the ICUs

The prevalence of bacteria and fungi were analyzed using PCR. The size distributions of each prevalent bacteria and fungi are shown in Figure 2. Of the 4 predominant bacteria, *Pseudomonas* spp. is a gram-negative bacterium that releases endotoxins. *Staphylococcus* spp. and *Micrococcus* spp. were found to be the highest at stage 4 (2.1-3.3 μm), while *Pseudomonas* spp. peaked at stage 5 (1.1-2.1 μm) and *Bacillus* spp. peaked at stage 1 (>7 μm).

The predominant fungi were *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp., and *Fusarium* spp. The *Cladosporium* spp., *Aspergillus* spp. and *Penicillium* spp. also showed peaks at the same stage 4 (2.1-3.3 μm) while *Fusarium* spp. showed a peak at stage 1 (>7 μm). Aspergillus fumigatus and Aspergillus flavus were found at $O_c$ and at $O_1$, while at $O_2$ only Aspergillus fumigatus was found. However, no *Aspergillus* spp. were found at the indoors of either the MICU or SICU.

## Discussion

### Indoor bioaerosol characteristics and size distributions

The difference of bioaerosol outdoor concentrations may be due to a better air flow (1.35±0.65 m/s with an ESE direction) at $O_2$, while, at $O_1$ and at $O_c$, a lower air flow (0.80±0.73 - 1.24±0.43 m/s) with a WSW direction passed through the construction site. Therefore the outdoor bioaerosol concentrations at $O_c$ were higher than $O_1$ and $O_2$. The higher bioaerosol concentrations in the SICU probably occurred from the limited space (80 $m^2$) which could not separate the SICU area into infection and non-infection areas as in the MICU (320 $m^2$). The activities of surgical treatments, such as wound care dressing and wound debridement, have the potential to spread bioaerosols into the environment.[16]

Normally the aerodynamic diameters of indoor bacteria in the clean environment ranged from 1 to 3 μm while indoor fungal spores ranged from 2 to 4 μm.[17] All of the bioaerosol size diameters were related to particles that are inhalable and suscep-

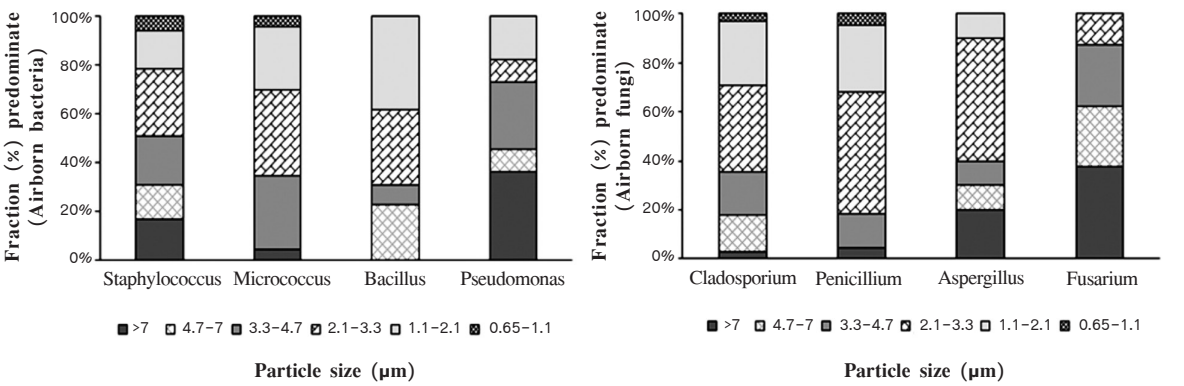


**Figure 2** Size distribution of fraction (%) predominant bioaerosols at the ICUs.

tible to deposit in the respiratory tract. These bioaerosols might be generated from droplet nuclei and infectious aerosols which were smaller than 2 and 5 μm, respectively.[1,2,18-20] The size distributions of fungi were similar to the results found in ambient fungi in the Wang et al.[21] study in China and in the study by Lin and Li.[22] In contrast, the results differed from what the Kim et al.[2] study found (i.e., the size distributions of fungi peaked in a range >7 μm). However, the different peaks of each microorganism especially in the SICU possibly was influenced by the UVGI system which controlled not only Mycobacterium tuberculosis[11,12] but also other microorganisms (Table 2 and 3).

There was little impact of the ambient air, especially from the construction site, that penetrated the MICU (I/O<1) and these ratios were also lower than the I/O ratio (2.1 times) reported by Kim et al.[2] The size distributions of bacteria were consistent with that of the Kim et al.[2] and Wang et al.[21] studies. From the wind direction in Figure 1, there was no influence of the construction site on the reference outdoor sampling for the SICU ($O_2$) and very low bacteria concentration (96.6±38.5 cfu/$m^3$). Therefore, the ratios of bacteria in the SICU, while the UVGI system was either turned on or off, were higher than the outdoor ratios (I/O>1) which was higher than the Kim et al.[2] study by about 2 and 4 times, respectively. However, the I/O ratio of bacteria in the MICU was lower than 1. Perhaps there was some contamination in the indoor environment that was due to the limitation of space in the SICU, occupant density and the low efficiency of the HVAC system.[2,19] However, Pastuszka et al.[23] found that a room with an HVAC system had a high level of bacteria and fungi size distribution in the ranges of 3.3-4.7 and 1.1-3.3 μm, respectively.

**Factors related to indoor bioaerosol concentration**

The total concentrations of indoor bacteria depended on UVGI usage (turn on and off) and room air velocity. The UVGI system was established in hospital areas to control infectious agents such as Mycobacterium tuberculosis.[11,12] However, the efficiency of the UVGI system depends on the relative humidity, indoor air velocity and room air circulation.[22] The appropriate effectiveness of the UVGI system with 42±19 μW/$cm^2$ upper-zone irradiance consisted of 50% RH, room size around 87 $m^2$, and 6 air changes per hour (ACH).[24] The outdoor fungi concentration, indoor air velocity, relative humidity and temperature significantly influenced the indoor fungi concentrations in the SICU. An increase in the indoor air velocity resulted in a decrease of indoor fungi concentrations. Even if the HVAC had a high-efficiency particulate absorption (HEPA) filter installed in this ICU, the overuse of the HEPA filter, the location of the ward doors[12], and anthropogenic source might have an influence on the indoor ICU fungi. The SICU had 2 conditions of UVGI on and off while the MICU had only one condition. While the UVGI was functioning, the indoor velocity and relative humidity were low and the indoor bioaerosols in the SICU had decreased by 2 times (Tables 1, 2, and 3).

**Predominant bioaerosols**

*Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp. and *Pseudomonas* spp. were the common bacteria found in this study and in other hospitals.[2,25] These results were different from the studies of Kim et al.[2] and Kim and Kim[20] which found that *Staphylococcus* spp. and *Micrococcus* spp. were identified most often at stage 5 (1.1-2.1 μm),

*Pseudomonas* spp. was found at stage 4 (2.1-3.3 μm) and *Bacillus* spp. was found at stage 1 (>7 μm). The sources of these bacteria in the hospital environmental conditions (e.g., tap water, sink drains, occupant density and patient bed preparation) should be more of a concern in order to lower their concentrations and avoid infection.[26,27] The predominant fungi were *Penicillium* spp., *Cladosporium* spp., *Aspergillus* spp. and *Fusarium* spp. These findings were consistent with other studies.[2,28-30] The *Aspergillus* spp. and *Fusarium* spp. can infect immunocompromised patients such as surgical, bone marrow transplant and cancer patients.[31-34] These fungi showed a peak at stage 4 (2.1-3.3 μm) while *Fusarium* spp. peaked at stage 1 (>7 μm). However, *Cladosporium* spp. and *Penicillium* spp. also showed a peak at stage 4 (2.1-3.3 μm). These results were in contrast to the Kim et al.[2] and Kim and Kim[20] studies that found *Fusarium* spp. was at both stage 1 (>7 μm) and at stage 3 (3.3-4.7 μm). These fungi genera are commonly found on the surfaces of armrests, beds, wash sinks, tables and medical devices, and their spores can spread easily indoors.[35-37] Allergies, inflammation and infections from these fungi genera are of considerable concern, especially for low-immunity patients with respiratory allergic symptoms and allergen sensitization.[38-41] In addition, there were no threshold limit values or cut-off levels for interpreting environmental measurements of bioaerosols for health and safety levels[42], but the results of Vonberg and Gastmeier[7], who reviewed nosocomial aspergillosis, found that the concentration of *Aspergillus* spp. below 1 cfu/$m^3$ can be enough to infect immunocompromised patients. To control the airborne infection based on the findings of this study, almost all of the predominate bioaersols were in the respirable size range and peaked at 2.1-3.3 μm. Only an HVAC with a HEPA filter may not be enough to filter the particulate matter smaller than 3 μm. In particular, the wet areas, anthropogenic source, air change per hour and indoor air velocity should be observed and monitored continuously.

## Conclusion

**The sizes of the bioaerosols in both ICUs were of respirable size. The MICU used a split HVAC system to control room temperature and the occupant density was lower; therefore, the airborne bacteria and fungi concentrations were lower than in the SICU. In the SICU, the central HVAC system with a HEPA filter and UVGI system were not enough to control the bioaerosols. The prevalent indoor airborne bacteria in both ICUs were *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp. and *Pseudomonas* spp. The prevalent indoor airborne fungi were *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Fusarium* spp. The concentrations and size distributions of bioaerosols in the ICUs were influenced by the bioaerosol species, location of sampling, indoor environment, HVAC with HEPA, UVGI, anthropogenic sources and the outdoor environment such as buildings that were under construction and meteorological factors such as air velocity, relative humidity and temperature. To decrease the indoor bioaerosol concentrations, the room air velocity should be increased and a UVGI system should be installed in the limited space of the ICUs. In particular, the wet areas, anthropogenic source, air change per hour, and indoor air velocity should be continuously observed and monitored.**

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