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Original Article

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Abstract

Exposure to bioaerosol in hospitals is associated with health effects but in the field of veterinary hospitals there are limited articles on bioaerosol concentration especially in Southeast Asia. This investigation of aerosol bacteria (cross-sectional study) was conducted in two veterinary teaching hospitals located in Thailand. Airborne bacteria were collected from air samples in four room types (reception hall, intensive care unit, out-patient department and in-patient hospital department) in each hospital using a sieve impactor air sampler at different periods (8:00–10:00, 10:00–12.00 and 13:00–15:00). The results revealed high bacterial contamination in all collected air samples. The average levels of total viable bacteria count were >500 colony forming units (CFU)/m^3 in all rooms but at some periods aerosol bacteria were <500 CFU/m^3. Also, in the late morning and afternoon period, aerosol bacteria increased from early morning period. Further investigation on the experimental efficacy of two different types of air purifier (also called cleaners in some of the literature) was tested to identify an alternative apparatus requiring limited space and competent for a high concentration of odor and animal fur. The non-ionized air purifier for animate space and the ozone generator air purifier for inanimate space significantly (p<0.05) reduced the aerosol bacterial concentration.

Keywords: Air cleaning, air pollution, air sampling, bioaerosol sampling, veterinary hygiene

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Introduction

Small-animal hospitals have a unique environment in which humans and animals share an enclosed space. This can lead to exposure to pathogens and allergens in this specific environment. The quality of air in the hospital environment has been a particular concern for the health of the hospital staff, visitors and small animals because the air in the hospital can serve as a medium for the transmission of infectious diseases (Francey *et al*., 2000; Obbard and Fang, 2003; Eames *et al*., 2009; Eickhoff, 2015). Pathogens in the air are distributed in a colloidal suspension formed by liquid droplets and particles of solid matter called a bioaerosol (Eames *et al*., 2009; Gizaw *et al*., 2016). Airborne infectious particles can be single-to-aggregated pathogenic cells or pathogens carried by other non-biological particles such as dust (Nevalainen *et al*., 1993; Hess-Kosa, 2011). The aerial pathogens reach target hosts on airborne particles from sources such as respiratory secretion from the mouths or noses of the hosts or other such sources, skin debris, mechanical ventilator circuits and air–conditioning plants (Parker, 1978; Richardson and Marples, 1982).

In a general indoor environment, the total bacteria count is one of the factors determining indoor air quality (Parker, 1978; Gröschel, 1980; Obbard and Fang 2003; Augustowska and Dutkiewicz, 2006; Harper *et al*., 2013). Air cleaning installations (also known as air purifiers) are generally recommended for indoor buildings as a part of environmental control measures. These installations consist of whole house-filtration room air cleaners (WHF) and free-standing room air cleaners (Sublett *et al*., 2010). WHF is a complex system with large portions of an interior air system including filters, such as high-efficiency particulate air (HEPA) and panel filters that must be installed with systems associated with central heating, ventilation and air-conditioning (HVAC). On the other hand, free-standing portable room air cleaners are preferable to WHF due their flexibility of movement within and between rooms (Sublett *et al*., 2010).

Ionizer air cleaners are one type of free-standing room air cleaner commonly based on ion emissions through a corona discharge producing ionization to decompose molecules in the air (Shiue *et al*., 2011). Ultraviolet (UV) light and ozone emissions are used in combination in ionizer air cleaners, where the UV light acts as a germicide and also produces hydroxyl radicals and ozone from oxygen molecules in ambient air (Medical Advisory Secretariat, 2005). The ozone released from the air cleaner then acts to destroy microbes as well as unpleasant odors in ambient air (Medical Advisory Secretariat, 2005). However, the ionic apparatus producing the ozone is designed for decontamination in unanimated spaces so the area must be ventilated before it is suitable for animate occupation. Electronic air cleaners (EACs) are another type of portable room air purifier that apply an electrical charge to eliminate airborne particles. Electrostatic precipitators (charged-media filters) based on pairs of oppositely charged plates trap particles when air passes through them (Agrawal *et al*., 2010). The non-ionizing electronic air filter type installed in EACs can reduce the risk of respiratory irritation and asthma symptoms due to the absence of ozone (Gent *et al*., 2003; Hood, 2005) and so is suitable for animate spaces.

There is currently limited knowledge on the importance of aerosol bacteria deposition in environmental contamination, especially in small-animal hospitals. The present study was conducted to gain knowledge of the quantity and variety of airborne microbes in the indoor air of two small-animal hospitals in Thailand during regular opening hours; and examined the impact of two types of free–standing portable room air cleaners based on changes in the aerosol bacterial load. The data obtained can be used for bioaerosol monitoring and the development of control measurement strategies in conventional animal hospitals where air filtration in the central air supply system is not possible due to limited space.

Materials and Methods

Sample sites: Four room types were sampled from two small-animal teaching hospitals in Thailand (designated as CAP and CEN in this study): 1) CAP, located in Bangkok with approximately 230,000 cases per year; and 2) CEN, located in central Thailand and ~100 km from Bangkok with approximately 50,000 cases per year. The types of rooms selected for airborne bacteria analysis were: reception/waiting hall (RH), intensive care unit (ICU), out-patient department (OPD), and in-patient hospital department (IPD). The RH samples were all located on the ground floor of the hospitals with a central air conditioner in CAP and natural ventilation in CEN. The ICU and OPD samples were all located on the ground floor of the hospitals with split-type, wall-mounted air conditioners in both hospitals. The IPD in CAP was located on the fifth floor with a central air conditioner, while the IPD in CEN was located on the ground floor with natural ventilation. The air conditioners in both hospitals were operated without special air filters.

Air sampling method and processing: An active air sampling method was selected for the airborne bacteria study using a sieve impactor (SAS Super ISO; VWR International PBI S.r.l., Milan, Italy) with an aspirated air flow of 180 l/min through a solid agar plate (nutrient agar supplemented with 10 mg/l cycloheximide). The impactor was sterilized prior to sampling and between measurements it was cleaned using 95% ethanol. The air sampler was set up in the center of the room at 1.2–1.5 m above floor level. For post sampling, the agar plates (BD Difco™ nutrient agar) containing cycloheximide were incubated at 37°C for 48 h to allow the growth of aerobic bacteria and then bacterial colonies were enumerated using CFU which were then converted to CFU/m^3.

Bioaerosol investigation: A cross-sectional study examined observational variables consisting of aerosolized aerobic bacteria during regular opening hours of the animal hospitals. In particular, aerial sampling was taken at three periods (8:00–10:00, 10:00–12.00, 13:00–15:00) from the room types (one sample plate/period/room). The hospital working hours normally included a short recess at noon for their staff,

so the air sampling excluded that 1 h period (12:00–13:00). Twelve sample plates (3 periods × 4 room types × 2 hospitals) were collected to evaluate bacteria.

Efficacy of air purifiers: Two types of commercial air purifiers (non-ionizing electronic and ozone generating) were tested for different room purposes. The non-ionizing electronic air filtration with an electrostatic field media filter was tested with rooms where there was activity 24 h a day. The ozone generator air purifier was used for rooms where there was not always activity or were closed during the night.

Experimental set-up for efficacy of air purifiers: In CEN, the experimental efficacy of air purifiers was tested early in the morning in ICU and OPD. Prior to the start of the experiment, the regular daily cleaning program of the hospital (surface cleaning on furniture and floors) was carried out. Control samples were then taken. Then a non-ionizing electronic purifier was run for 1 h and an ozone generator for 30 mins to decontaminate the air. In particular, the ozone-generator air purifier was switched off for 10 mins before the air sample was taken. In brief, bioaerosol bacteria were collected and incubated as described above. The air samples from un-purified air and purified air (before and after air-cleaning) were repeated 8–10 times for each air-cleaning method. The levels of CFU/m^3 were calculated. The number of air samples was calculated with expected difference between mean = 90, expect standard deviation = 30, level of confidence 95%, a power of 80% so the inclusion of ≥ 4 samples was chosen in each group of study (before and after the air-cleaning group). Then, the bacterial loads from untreated air and treated air were tested using either a paired two-sample t-test or the Wilcoxon signed-ranks test at the 95% confidence interval (CI). The study protocol was approved by the Scientific Research Committee, Kasetsart University, Thailand. The authors declare there were no conflicts of interest within this study.

Results

Bacteria discovered from aerosol investigation: CAP had a lower bacterial count than CEN (Fig. 1). The cumulative bacterial count from the tri-periods in CAP was 10,290 CFU/m^3 compared to 14,825 CFU/m^3 in CEN. The study indicated that, overall, the highest total bacteria count was from ICU in CEN (8,675 CFU/m^3). In CAP, the average aerosol bacteria count from IPD, RH, OPD, and ICU were 510, 830, 695, and 1,395 CFU/m^3, respectively. The average bacteria counts in each room of CEN were 550, 630, 870 and 2,892 CFU/m^3, respectively. Overall, the highest bacteria count (3,730 CFU/m^3) during 10:00–12:00 was from ICU in CAP. Trends of bioaerosol within sampling periods are shown in Fig. 2 which indicates that the bacterial concentrations in the early morning period (8:00–10:00) were generally lower than in the late afternoon period (13:00–15:00) in both hospitals, especially in IPD and OPD.

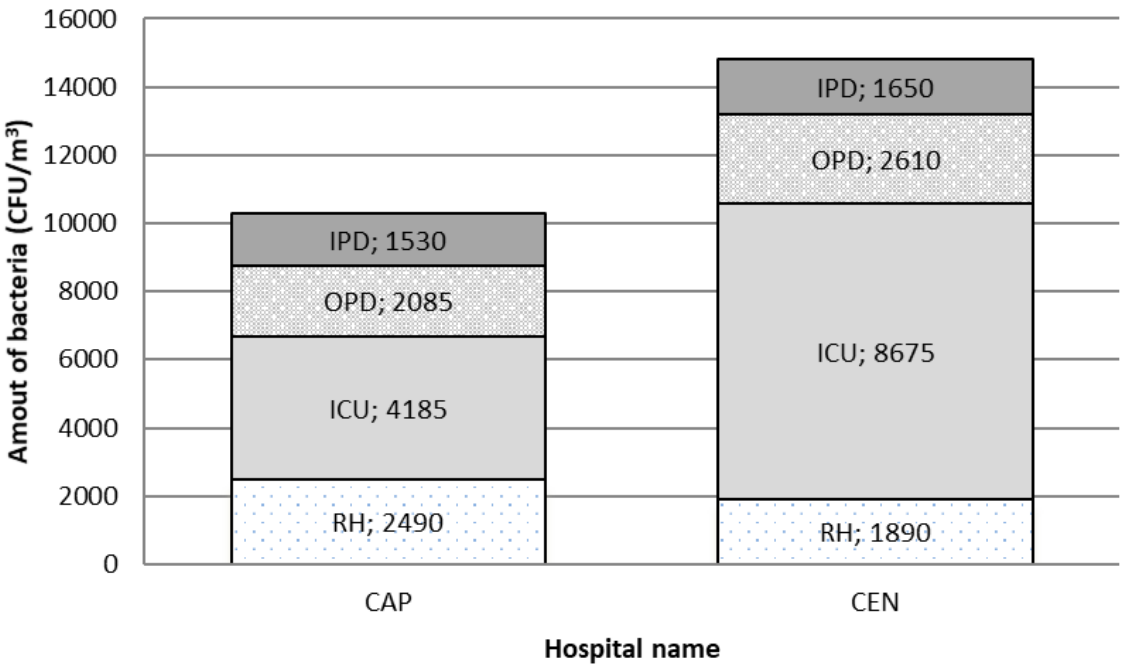

Figure 1 Cumulative data on aerosol bacteria from three periods in the two small-animal hospitals, Thailand (RH: reception/waiting hall, ICU: intensive care unit, OPD: out-patient department, IPD: in-patient department)

Efficacy of air purifiers: The quantitative analysis was mainly conducted to determine the number of bacteria in the indoor air from the two types of air purifiers (Table 1). The performance of the non-ionized air purifier was tested against 24 h cycle usage rooms. The results showed that there was a difference between the means of bacteria from un-purified air and purified air. With paired two-sample t-test, the electronic air filter significantly changed the bacterial load (p-value = 0.0347) at 95% CI. The average proportion of microbial

load between un-purified air and purified air using the non-ionized air purifier was 6.32.

The efficacy of the ozone generator air purifier was tested against the room type with a resting period between the 10 replicates. The ozone generator air purifier was able to kill microorganisms in the indoor air by reducing the microbial load by 2.27 times. Using the Wilcoxon signed-ranks test, the ozone generator air purifier significantly reduced aerosol bacteria (p-value = 0.0273; 95% CI).

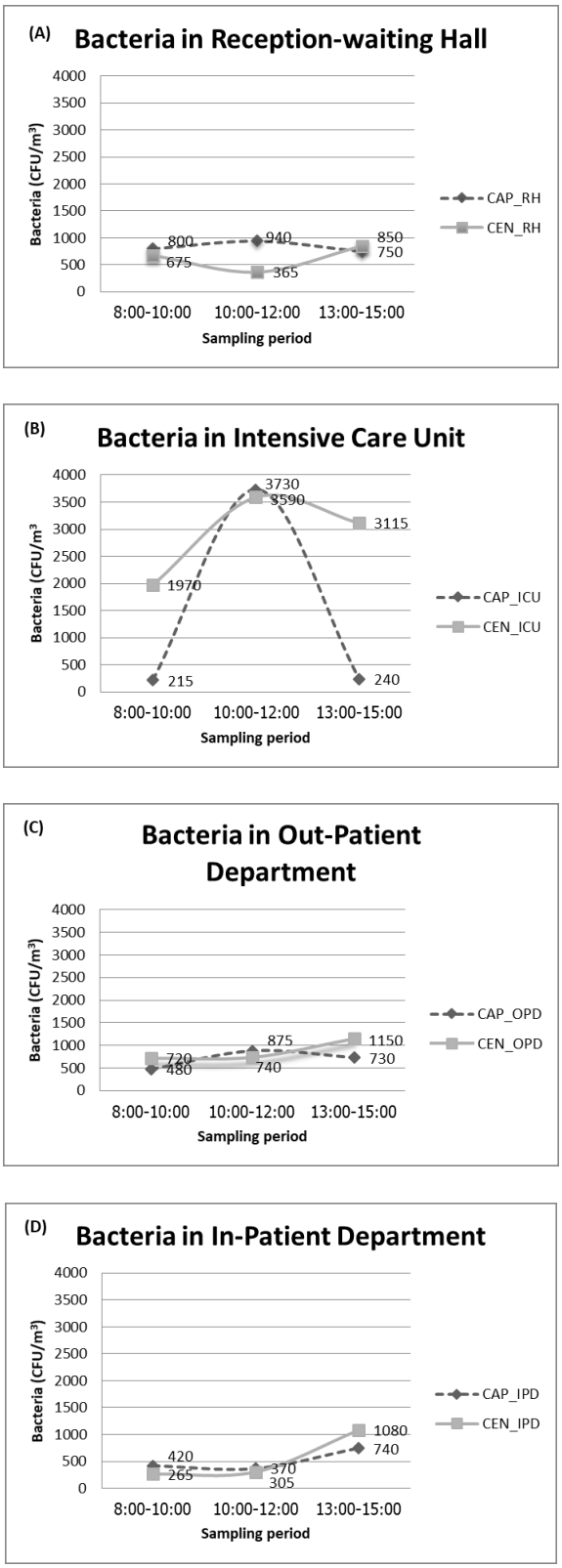

Figure 2 Bacterial concentration (CFU/m³) of indoor air of small-animal hospitals according to sampling period (8:00–10:00, 10:00–12:00 and 13:00–15:00)

Table 1 Descriptive data on the effect of air cleaning methods on bacteria loads (CFU/m^3)

Air cleaning methods	Mean	SD	95 % Confidence interval for mean	
			Lower bound	Upper bound
Non-ionized air purifier				
• Un-purified air	543.75	480.27	142.23	945.27
• Purified air	115.00	82.07	46.39	183.61
Ozone generator				
• Un-purified air	374.5	419.81	74.19	674.81
• Purified air	193.5	190.16	57.47	329.53

Discussion

Air-borne transmission is one of the causes of hospital-acquired infections. An approximate 10–20% of all endemic nosocomial infections are caused by air-borne transmission (Eickhoff, 2015), and the impact of inferior air quality in hospitals may lead to hospital-acquired infections, sick hospital syndrome and occupational risks (Pitarma *et al*., 2017). Many researchers have become progressively interested in the increased morbidity and mortality associated with nosocomial infections in ICU (Vincent *et al*., 1995; Jaffal *et al*., 1997; Inweregbu *et al*., 2005) and the ICU has been reported to have the highest prevalence of hospital-acquired infections in a hospital setting (Inweregbu *et al*., 2005; Kouchak and Askarian, 2012). The average airborne bacterial load from ICUs in the current study (2,143.3 CFU/m^3) was 8.6 and 3.2 times higher than the average microbes from the ICU of a (human) tertiary care public hospital (248.9 CFU/m^3) (Chuaybamroong *et al*., 2008) and from a human hospital in Abu-Dhabi (660 CFU/m^3) (Jaffal *et al*., 1997), respectively. The ventilation system in the tertiary care public hospital mentioned was a central air system equipped with a series of filters (prefilter, medium filter, HEPA filter) (Chuaybamroong *et al*., 2008) while the ICU in the Abu-Dhabi case had high sanitary standards (Jaffal *et al*., 1997). These might be reasons why the total bacteria counts were lower than in animal hospitals.

Normally outdoor bacteria are more diverse than in indoor environments due to the greater external diversity of bacterial sources (Fujiyoshi *et al*., 2017). Previous observations indicated an association between outdoor air particles and indoor air particles (Mohammadyan and Shabankhani, 2013; Soleimani *et al*., 2016). The influence of outdoor air on microbial level change might be due to the air exchange between indoors and outdoors through the main hospital entrance (Park *et al*., 2013). Hospital lobbies and emergency units with an entrance connected to the outdoor environment are likely to increase the chances of exposure to a variety of bioaerosol (Luksamijarulkul *et al*., 2004; Park *et al*., 2013). Specific diseases such as Legionnaires' disease are indicated as having an association with the OPD but not with other parts of the hospital (O'Mahony *et al*., 1990). Increasing the amount of good quality outdoor air coming indoors is another approach to lowering the concentrations of indoor air pollutants (United States Environmental Protection Agency, n.d). In the RH of CAP, air circulation between the indoors and outdoors affected microbial fluctuations by natural ventilation through doors while bacterial concentrations of RH-CEN were truly dependent on outdoor air bacteria. The RH of CEN was not the installed air conditioner and its ventilation was only on natural ventilation.

In the present study, daily fluctuations of airborne microflora were found in the animal hospitals. The first period of sampling (8:00-10:00) seemed to have a low microbe concentration while after the mid-day testing periods (the second and third periods of sampling), the indoor air microorganisms increased in number. The study found that morning had lower aerosol bacterial population counts than the afternoon which was different from previous research (Augustowska and Dutkiewicz, 2006; Harper *et al*., 2013; Park *et al*., 2013). There were issues concerning sources of airborne nosocomial infection (Schaal, 1991; Eickhoff, 2015) as being: 1) from inside the hospital via humans (patients, health care workers, visitors), air particles (infective dusts, aerosols) and ventilation or air-conditioning systems; 2) from outside the hospital via soil, water (cooling towers), decaying organic material and dust from construction. The human occupancy level was interpreted as a major element that affected the concentration of indoor air bacteria in several healthcare facilities (Li and Hou, 2003; Chuaybamroong *et al*., 2008; Park *et al*., 2013; Eickhoff, 2015; Kumar *et al*., 2018) as well as in university libraries (Hayleeyesus and Manaye, 2014). The presence of pets (dogs and cats) is one of the factors influencing the increase in aerial microbial flora in the community (Kettleson *et al*., 2015; Scherer *et al*., 2016). So elevated occupancy level and increasing air particles in the afternoon of this study might have influences on high aerosol bacterial population counts in the afternoon.

Level of aerosol bacteria in the ICUs seemed to have a major impact on bacteria counts in the two small-animal hospitals in the study (Fig. 1). Without ICUs, cumulative bacterial count from the tri-periods from CAP and CEN were fairly similar (6,105 and 6,150 CFU/m^3 respectively). CAP had a higher number of cases than CEN but ICU-CAP had lower bacteria counts than ICU-CEN. Researchers revealed that high bioaerosol concentrations in community hospitals were found in the emergency unit as well as in the OPD (Luksamijarulkul *et al*., 2004; Cabo Verde *et al*., 2015). The ICU-CAP was located on the ground floor of the hospital and was connected with the emergency unit through a sliding door. The ICU-CEN was also located on the ground floor and the room size was close to ICU-CEN and was designed for use in two functions as an ICU and an emergency unit. In this study, it is suggested that the indoor bioaerosol population might be elevated in the ICUs because of the hospital layout and the presence of pets. Humidity and ventilation issues from CEN building structure and additional functions (emergency task) of ICU-CEN such as wound lavage, intubation, hair clipping and resuscitation were potential factors to generate aerial

bacteria, which might be reasons for the especially high bacteria count (>1,900 CFU/m^3) from the tri-periods in ICU-CEN. The peaks of bacterial concentration in ICUs were at the second period of sampling (10:00–12.00) in Fig. 2B. The comprehensive activities accommodating more people might be reasons for the remarkably increasing level of aerosol bacteria in the ICUs during the second period of sampling in this study involving physical activities such as changing shift patterns, admitting and transferring cases and case consultations with specialists. However, the present study was a cross-sectional study suggesting more sampling on frequency should be performed in future research.

Air-control measures are crucial for reducing the dissemination of air bioburden in hospitals. The indoor aerosol bacteria counts from the present study were almost all higher than the recommended air level-the guidelines of the American Conference of Governmental Industrial Hygienists (ACGIH) (> 500 CFU/m^3) (Seitz, 1989) except for the samples from the IPDs in both hospitals (during 8:00–10:00 and 10:00–12:00) and from the ICU in CAP (during 8:00–10:00 and 13:00–15:00), which indicated generally poor ventilation or unhygienic conditions. Contaminant source reduction and sufficient air exchange rate are the basics in environmental control strategies (US Environmental Protection Agency, 1991; Fox 1994). Source reduction such as restricting visitor numbers, limiting the amount of material (flowers, food) brought in from outside and changing to more use of hospital gowns must be regularly applied in all areas in a hospital. Beside such administrative controls, another indoor air environmental control strategy includes the use of air cleaning devices adjunctively with other environmental controls to achieve additional reduction of airborne biological particles (Fox, 1994). The engineering methods for indoor air bioaerosol control are usually carried out by the building's HVAC system by diluting (dilution ventilation) and controlling airflow direction in a building (Fox, 1994). Inadequate maintenance of the HVAC system over time may limit its ability to maintain acceptable air quality. Likewise, the budget for maintenance, other limitations related to installing HVAC in an old building were often due to inappropriate sizes of the systems (normally too large) to be installed and weaknesses in the existing building structure that may result because of the weight and vibrations during operation. In-room air cleaners supplied as portable or fixed units have been recommended as alternative choices to HVAC systems (Bozzi *et al*., 1994; Miller-Leiden *et al*., 1996; Jensen *et al*., 2005).

Filters in an air cleaner and in an air conditioner are important keys in the control of microbial concentrations (Chuaybamroong *et al*., 2008). Unsurprisingly, several studies have indicated an effective reduction in air bacterial concentration on the HEPA filter for the indoor air in hospitals through using mechanical filtration (Medical Advisory Secretariat, 2005; Charkowska, 2008; Chuaybamroong *et al*., 2008; Cabo Verde *et al*., 2015) because HEPA can clear 90% of particles larger than 0.3μm in diameter(Medical Advisory Secretariat, 2005). To be fully effective, HEPA filters must be leak-proof and have scheduled maintenance to replace new filters (recommended annually) but this involves higher maintenance costs compared to other types of filters and high dust contaminated areas may need more regular maintenance than once a year. Additional pollutants such as gases, fumes, chemicals and odors cannot be trapped by an HEPA filter. In animal hospitals and clinics, common environmental problems are odors, infectious pathogens and aerosol dust. This present study aimed to identify an optional, cost-effective, air cleaning system that could be used in general animal clinics while requiring limited space for installation. The results from the air purifying experiment in this study indicated that the two different types of air cleaner could be used in actual animal hospital rooms with suitable effectiveness. These results suggest in-room air purifiers for hospitals having space limitations using a non-ionizing electronic purifier for animate space and a UV/ozone generator for non-animate space. The frequency of cleaning and hospital ventilation should be adjusted based on operating hours and the number of occupants in an area. For example, according to the study results, air purifiers in animate spaces should operate in IPDs and ICUs from late morning (10:00–15:00) with maintenance (non-operation) in early morning, while in inanimate spaces (the RH of CAP and OPDs), air cleaning should be scheduled during the night and at dawn (before 05:00).

Air particle dust is also an important consideration regarding airborne diseases. Floor cleaning or maintenance activities during hospital service hours had an influence on escalating airborne levels (Park *et al*., 2013). Floor cleaning can contribute to increased levels of airborne microorganisms because of the mechanism of particle dispersal from the floor into the air. Floor sweeping is commonly practiced for cleaning purposes in animal hospitals using a broom, which can stir up dust and add dust to the surrounding air. In addition, vacuum cleaners with HEPA filters should be used for cleaning to remove dry floor particles in order to prevent microbial aerosolization from the floor. In addition, cleaning times should be scheduled after the end of the day shift or during a low activity period.

A major limitation of this cross-sectional study was the short sampling times, with only one sample in each period, so the results may not be representative of the complete year. The bacterial concentration also varied during different times of the day (Dharan and Pittet, 2002). Other environmental factors that could be examined include: temperature, humidity, and the number of occupants in a room, as these might influence the level of airborne microorganisms in animal hospitals. Nevertheless, this study could be useful for identifying bioaerosol problems in small-animal hospitals especially in Thailand and Asia and also to develop effective measures to reduce microbial load. Based on our results, engineering control, administrative control and personal protective equipment should be used where appropriate to reduce exposure to microorganisms in small-animal hospitals. Further study of airborne bacteria in animal hospitals in Asia should include operating theater rooms and a study of type variation in the bacteria. Based on the present study, sampling times should be

at the peak of microbial load (10:00–12:00 or 13:00–15:00) in a normal operational day.

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