ASSESSMENT OF INDOOR AIR QUALITY AND PARTICLE SIZE DISTRIBUTION OF TOTAL BACTERIA AND *STAPHYLOCOCCUS* SPP IN AN URBAN HOSPITAL IN THAILAND

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Abstract. Indoor air quality (IAQ) and particle size distribution of total bacteria and *Staphylococcus* spp in an urban hospital, Thailand were conducted in five outpatient departments using an Andersen six-stage impactor, together with measurements of indoor temperature, relative humidity and carbon dioxide level. Total airborne bacteria and *Staphylococcus* spp concentration ranged 75-2,261 and 12-292 CFU/m^3, respectively, with *S. aureus* concentration in the range 0-7.1 CFU/m^3. Mean airborne bacterial concentrations at four sampling locations (738±867, 533±372, 689±528 and 551±474 CFU/m^3 for locations A, B, C and D, respectively) were all above the acceptable standard of 500 CFU/m^3. In contrast, the mean airborne bacterial concentrations at location E (391±313 CFU/m^3) did not exceed the acceptable standard. There was no association between bacterial concentration and physical parameters measured, but it was noted mean CO_2 level at almost all of the sampling locations was above acceptable standard (1,000 ppm). The particle size distribution demonstrates that 75% of total bacteria and 55% of *Staphylococcus* spp was in the size range (aerodynamic diameters below 4.7 μm) capable of being deposited in the lower respiratory tract. High airborne bacterial concentration and CO_2 level were indicative of poor ventilation, overcrowding and unsanitary IAQ. Presence of respiratory system microorganisms poses high risks infection of vulnerable patients. These findings highlight the urgent need to rectify this situation and to implement policies for improving and maintaining proper indoor air quality in the studied locations. This type of survey should be carried out on a regular basis in all hospitals across the country.

Keywords: *Staphylococcus aureus*, bacteria, bioaerosol, hospital, indoor air quality, outpatient department, particle size distribution

INTRODUCTION

Hospital being a public place facility is commonly associated with continuous and complex exposure to patients and healthcare workers of airborne hazards of different types, such as allergens, infectious agents, particulates, and toxic compounds (Li and Hou, 2003; Ortiz *et al*, 2009; Bolookat *et al*, 2018; Luksamijarulkul *et al*, 2019). Improper control of hospital indoor air quality (IAQ) can result in

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nosocomial infections and occupational diseases. Malfunction and contamination of ventilation systems increase risk of spread of airborne microorganisms or bioaerosols linked to outbreaks of nosocomial infections (Uduman *et al*, 2002; Rodrigo *et al*, 2018).

Bioaerosol, considered an essential component of IAQ assessment, is defined as a broad category of airborne particles containing living organisms (Ghosh *et al*, 2015), bacteria being the predominant infectious microorganism in hospital wards, *viz Acinetobacter* spp, *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant *Enterococcus* spp (Weber *et al*, 2013; Dancer, 2014; Ling and Hui, 2019). In general, bacterial cell ranges 0.5-10 μm in length and the microorganism is able to be present in aerosol as a single cell, aggregate of several cells or bound on biological and non-biological particle (Polymenakou *et al*, 2008). Ability of bioaerosol to penetrate human respiratory system depends on its particle size: small aerosol particle (<5 μm) can accumulate in bronchioles and alveoli of the lower respiratory tract, remaining for long periods before being removed by macrophages; larger aerosol particle (>7 μm) resides in the upper respiratory tract where it can cause an allergic reaction and, if carrying viable pathogens, gives rise to infection (Lindsley *et al*, 2017). Bacterial count is significantly correlated with number and size of airborne particles (Tham and Zuraimi, 2005) and so is the converse (Hansen *et al*, 2005).

Prevalence of a single *Staphylococcus*- and/or multiple *Staphylococci*-laden airborne particles in a hospital environment should be considered an immediate concern as *Staphylococcus* spp are considered important human pathogens, with *S. aureus* being responsible for major outbreaks of nosocomial infection (Botelho *et al*, 2012; Gizaw *et al*, 2016; Luksamijarulkul *et al*, 2019). *Staphylococcus* spp have the ability to survive for long periods on dry and inanimate surfaces (Kramer *et al*, 2006). *S. aureus* is capable of colonizing the nasal cavity and bronchi, eventually causing nasal infection and pneumonia (Kaye *et al*, 1990; Corne *et al*, 2005). Moreover, these bacteria tend to bind to particulate matter and are prevalent in interior spaces with poorly maintained or malfunctioning air conditioning systems (Ling and Hui, 2019).

In order to protect patients and healthcare workers from nosocomial infections, particular attention is required to ensure healthful IAQ in a complex hospital environment. Knowledge of concentration and particle size distribution of bioaerosols at various hospital interior locations is critical in predicting their risk to nosocomial infections in patients and healthcare workers (Nasir *et al*, 2005). Thus, this study was conducted to assess IAQ and airborne particle size distribution of total bacteria and *Staphylococcus* spp concentration in outpatient departments of a hospital to obtain a more complete understanding of their risks in causing nosocomial infection, thereby leading to development of improved and effective control strategies.

MATERIALS AND METHODS

Sampling sites

A cross-sectional study was carried out in waiting-area of five outpatient departments, namely, Department of Allergy, Asthma and Pulmonary Disease (location A), Department of Pediatrics (location B), Department of General Medicine-1 (location C), Department of

General Medicine-2 (location D), and Department of Obstetrics and Gynecology (location E) of a hospital in Pathum Thani Province, Thailand (Fig 1). These outpatient departments are the busiest places of the hospital. At each location, microbiological samples were taken three days a week (Wednesday, Friday, and Saturday) to cover days with maximum (Wednesday) and minimum (Saturday) numbers of patients. These sampling dates were selected according to the monthly record of the patient number provided by the Medical Record Service of the hospital.

Research protocols were approved by the Biosafety Committee of Thammasat University, Thailand (no. 001/2562). Use of microbiological agents was in strict accordance with recommendations in the Biosafety Guidelines for Work Related to Modern Biotechnology or Genetic Engineering of Thailand's Technical Biosafety Committee.

Bacterial aerosol sampling and physical parameters measurement

A pilot study was performed to evaluate the appropriate air sampling volume and sampling period (data was not shown). Bacterial aerosol concentration was measured in the morning using an Andersen six-stage impactor with a volume of 28.3 l/min for 15 minutes (BGI Inc, Waltham, MA). The biosampler has particle cutoff diameters (d_{50}) of 7.0 μm (STG1), 4.7 μm (STG2), 3.3 μm (STG3), 2.1 μm (STG4), 1.1 μm (STG5), and 0.65 μm (STG6), and placed at a height of 1.5 m above ground level to access air at the height of human breathing zone. Before each sampling experiment, the impactor was sterilized and equipped with trypticase soy agar (TSA) (Oxoid, Hampshire, UK) and Baird Parker agar (BPA) (Oxoid, Hampshire, UK) for culturing total bacteria and *Staphylococcus* spp respectively. At the same time, measurements of temperature, relative humidity (RH) and CO_2 concentration were monitored for 60 seconds using an IAQ-Calc™ Indoor Air Quality Meter 7525 (TSI Inc, Shoreview, MN).

Microbiological assays

Following completion of air sampling experiment, TSA and BPA were incubated at 37°C for 48 hours. Colonies on TSA were counted as aerobic mesophilic bacteria. Grey-black shiny convex colonies on BPA, 1-1.5 mm in diameter with narrow entire white margin surrounded by a clear zone, were suspected as *Staphylococcus* spp, and confirmed by Gram's staining (Foster, 1996). *S. aureus* colonies were identified by metabolic characteristics and production of catalase, coagulase, and DNase in the presence of mannitol (Foster, 1996).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program version 18 for Windows (PASW serial no. 5082357) (SPSS Inc, Chicago, IL). Data were analyzed using descriptive statistics, *eg* frequency distribution, percentage, and mean ± standard deviation (SD). Correlation among parameters (temperature, RH and CO_2 concentration) and microbial IAQ was evaluated using a Pearson's coefficient with a statistical confidence level = 0.05. Kruskal-Wallis test was employed when comparing among bioaerosol concentrations of different size ranges in the five sampling sites.

RESULTS

Work activities and air conditioners installed at the hospital five study locations

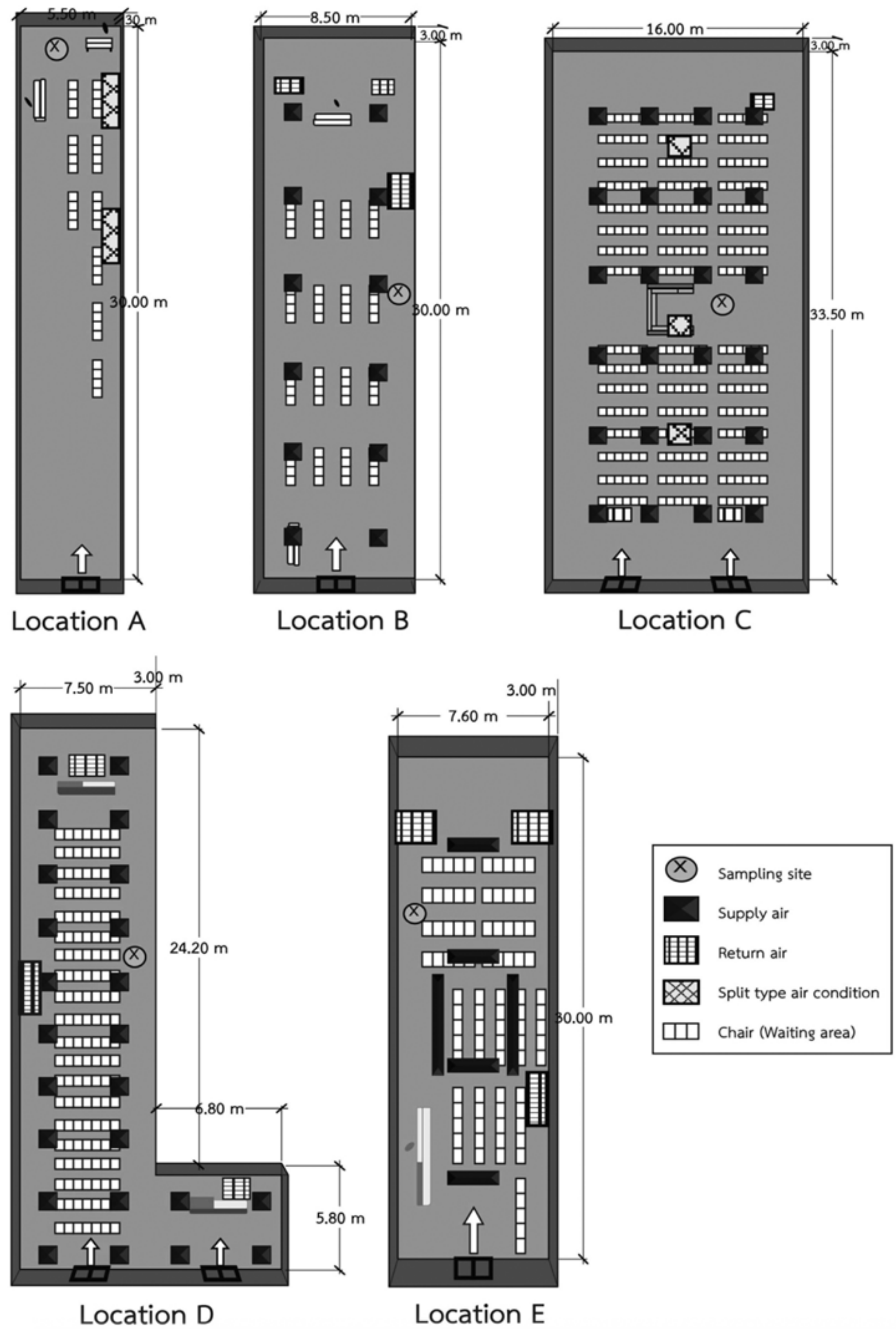

Fig 1-Map of waiting rooms at a hospital in Pathum Thani Province, Thailand where bioaerosol samples were collected.

Location A: Department of Allergy, Asthma and Pulmonary Disease; Location B: Department of Pediatrics; Location C: Department of General Medicine-1; Location D: Department of General Medicine-2; Location E: Department of Obstetrics and Gynecology.

Table 1 presents patient and healthcare staff's general activities in the waiting rooms of five study out-patient departments, including movement, waiting, and registration. The occupant density ranged from 0.7 to 72.2 people/1000 feet^2, while the highest occupant density was observed in location E (Department of Obstetrics and Gynecology). Recirculating air conditioning system was applied in almost locations except for location A (Department of Allergy, Asthma and Pulmonary Disease), where split-type air conditioning system was installed. The temperatures at locations A, C (Department of General Medicine-1) and E (Department of Obstetrics and Gynecology) were slightly above the guidelines range (20.0-25.5^{o}C) (ASHRAE, 2010a). CO_2 concentration at locations A, B (Department of Pediatrics), C and D (Department of General Medicine-2) was higher than the acceptable IAQ limit (1,000 ppm) (ASHRAE, 2010b) (Table 2).

Airborne bacteria and *Staphylococcus* spp concentrations at the five study hospital locations

Mean total airborne bacteria concentrations collected at waiting room of the five study out-patient departments ranged from 75 to 2,261 CFU/m^3, highest at location A (allergy, asthma and pulmonary disease department) and lowest at location E (obstetrics and gynecology department), with mean concentration at all locations except location E (Table 2) higher than the acceptable IAQ limit of 500 CFU/m^3 (ACGIH, 1999). *Staphylococcus* spp were collected at all locations, highest mean concentration being at location C and lowest at location E (Table 2). Mean *S. aureus* concentration collected at four locations was 0.4 ± 1.3 CFU/m^3 (range: 0 - 7.1 CFU/m^3). Mean *Staphylococcus* spp concentration was positively correlated with RH in locations B ($r^2 = 0.785$, p-value <0.050) and D ($r^2 = 0.783$, p-value <0.050).

Particle size distribution of airborne bacteria and *Staphylococcus* spp at the five study locations

Employing an Andersen six-stage impactor (BGI Inc, Waltham, MA) to determine particle size distribution at the five study locations, the majority of bacteria was collected at STG5 (d_{50} = 1.1 μm) (Fig 2) and the proportion of bacteria that could lodge in the upper ($d_{50} \geq 4.7$ μm) and lower respiratory tract (d_{50} <4.7 μm) was 25 and 75% respectively. The majority of *Staphylococcus* spp was collected at STG1 (d_{50} = 7.0 μm) and the proportions of *Staphylococcus* spp with the possibility in the upper and the lower respiratory tract were 45 and 55%, respectively. Difference between concentration of bacteria and *Staphylococcus* spp at different particle size range is statistically significant (p-value <0.050, Kruskal-Wallis test).

DISCUSSION

Because indoor air quality (IAQ) is of utmost concern for occupational and public health and exposure to indoor air pollutants can increase people's risks of developing adverse health effects, IAQ and particle size distribution of airborne bacteria and *Staphylococcus* spp were assessed in five outpatient departments of a hospital in Pathum Thani Province. The findings show concentrations of total bacteria obtained from four sampling locations are above acceptable standard recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1999). In addition, the presence of *Staphylococcus* spp, in particular *S. aureus* (0.07-0.24% of *Staphylococcus* spp) creates an additional risk as these bacteria can cause nosocomial

Table 1
Location and activity in the waiting rooms at a hospital in Pathum Thani Province, Thailand where bioaerosol samples were collected.

Location (Department)	Air conditioning system	Activity	Room area ($feet^2$)	Occupancy density (no. of people/1,000 $feet^2$)
A	Split-type air conditioner	Patient movement and waiting for medical examination	1,776	2.8-26.5
		Healthcare staff movement, patient registration and other activities		
B	Recirculating air conditioner	Patient movement and waiting for medical examination	2,745	3.3-11.7
		Healthcare staff movement, patient registration and other activities		
C	Both split-type and recirculating air conditioners	Patient movement and waiting for medical examination	5,769	21.0-35.5
		Healthcare staff movement, patient registration and other activities		
D	Recirculating air conditioner	Patient movement and waiting for medical examination	2,846	0.7-52.7
		Healthcare staff movement, patient registration and other activities		
E	Recirculating air conditioner	Patient movement and waiting for medical examination	1,366	36.6-72.2
		Healthcare staff movement, patient registration and other activities		

A: Department of Allergy, Asthma and Pulmonary Disease; B: Department of Pediatrics; C: Department of General Medicine-1; D: Department of General Medicine-2; E: Department of Obstetrics and Gynecology.

Table 2
Airborne bacteria and *Staphylococcus* spp concentrations and environmental parameters of outpatient waiting rooms at a hospital in Pathum Thani Province, Thailand.

Indoor air quality parameter	Location				
	A	B	C	D	E
Total bacteria (CFU/m^3)					
Mean (SD)	738 (867)	533 (372)	689 (528)	551 (474)	391 (313)
Range	127-2,261	165-1,003	212-1,435	75-1,204	97-839
Staphylococcus spp. (CFU/m^3)					
Mean (SD)	96 (103)	95 (66)	149 (39)	87 (53)	42 (28)
Range	12-292	26-210	111-198	16-162	14-92
S. aureus (CFU/m^3)					
Mean (SD)	1.7 (2.5)	0.1 (0.6)	0.1 (0.6)	Undetectable	0.1 (0.6)
Range	0-7.1	0-2.4	0-2.4	-	0-2.4
Temperature (°C)					
Mean (SD)	27.0 (0.3)	25.0 (2.9)	27.0 (1.1)	24.0 (2.6)	26.0 (9.2)
Range	26.8-27.0	22.1-28.5	25.3-27.6	21.0-26.8	26.0-26.5
Relative humidity (%)					
Mean (SD)	65 (5)	56 (4)	58 (4)	61 (5)	51 (2)
Range	61-72	51-61	52-60	55-65	48-54
CO_2 (ppm)					
Mean (SD)	1,248 (538)	1,794 (420)	1,022 (381)	2,038 (1,015)	827 (70)
Range	553-1,613	1,278-2,196	556-1,392	742-2,859	771-916

A: Department of Allergy, Asthma and Pulmonary Disease; B: Department of Pediatrics; C: Department of General Medicine-1; D: Department of General Medicine-2; E: Department of Obstetrics and Gynecology; SD: standard deviation; CFU/m^3: colony forming units per cubic meter; ppm: part per million.

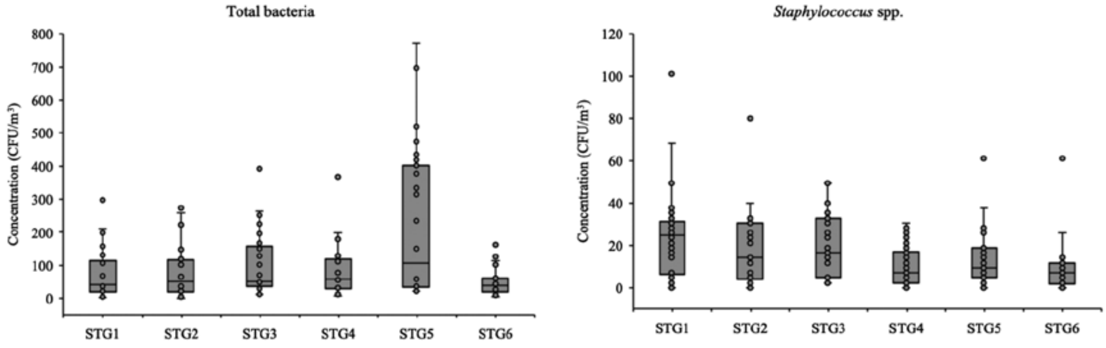

Fig 2-Boxplots of total bacteria and *Staphylococcus* spp concentration (CFU/m^3) collected using an Andersen six-stage aerosol collector placed in five outpatient waiting rooms of a hospital in Pathum Thani Province, Thailand.

Each circle represents outlier points and inner points. Within each box, the inside line represents the median and the lower and upper hinges of each box represent the 25th (Q1) and 75th (Q3) percentiles, respectively, while the lower and upper whiskers represent the minimum and maximum values.

STG1: d_{50} = 7.0 µm; STG2: d_{50} = 4.7 µm; STG3: d_{50} = 3.3 µm, STG4: d_{50} = 2.1 µm; STG5: d_{50} = 1.1 µm; STG6: d_{50} = 0.65 µm.

infections leading to a variety of clinical infections, such as of skin and pulmonary system, and if systemic can be life-threatening (Kramer *et al,* 2006; Tong *et al,* 2015).

In general, accumulation of bioaerosols in buildings primarily arises from outdoor air introduced by air exchange and indoor emission from occupants and type and use of buildings (WHO, 1990). In hospitals, occupant density and activity are the main determinants of airborne bacteria level (Obbard and Fang, 2003; Pastuszka *et al*, 2005; Park *et al*, 2013), but temperature, RH, particle size, and particle composition are also physical factors contributing to airborne bacteria survival (Lighthart and Shaffer, 1997). In the current study, no correlation was discerned between airborne total bacteria concentration and measured physical parameters, namely, temperature, RH and CO_2 concentration. However, CO_2 levels at location A, B, C and D were above the guideline range recommended by the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE, 2010b). CO_2 is introduced as an IAQ representative of indoor air pollutants that may cause residents to become tired, have stagnant movement, suffer from headache, or function at lower activity level (Cincinelli and Martellini, 2017). In addition, CO_2 is a surrogate parameter for indoor air pollutants emitted by residents when it correlates with human metabolism activity (Niemelä *et al*, 2017; Kapalo *et al*, 2019). The maximum occupant densities in waiting rooms at the study hospital were higher than default value recommended by ASHRAE (2001), a hospital waiting area and lounge of 10 people/1000 feet^2, indicating insufficient ventilation due to use of recirculating air conditioners. Obbard and Fang (2003) noted occupant density is a critical factor influencing airborne bacteria concentration in a hospital in Singapore.

A positive correlation between mean airborne *Staphylococcus* spp concentration and RH was found in waiting rooms of Departments of Pediatrics and Departments of General Medicine-2. Andualem *et al* (2019) noted bacterial load in indoor air of public primary schools is associated with RH. Air moisture changes bacteria cell wall integrity and an increase in air humidity promotes bacteria growth, whereas a dry environment decrease metabolism and physiological activities of microorganisms (Jones and Harrison, 2004). On the other hand, Thompson *et al* (2011) found RH does not affect survival of staphylococci in aerosols. These discrepancies may be due to species diversity, environmental quality, sampling technique, and culture method employed (Harper *et al*, 2013; Chaivisita *et al*, 2018). In healthcare settings, contact and droplet transmission are the principal routes of *S. aureus* transmission. For airborne transmission, acquisition of nasal carriage and contaminated skin squamae are observed in patients and healthcare workers (Solberg, 2000; Beggs, 2003).

Particle size is a crucial parameter influencing the result of human exposure to airborne microorganisms (Morawska, 2006). Overall, the current study demonstrates that 75% of total bacteria and 55% of *Staphylococcus* spp had size ranges capable of being deposited in the lower respiratory tract, including bronchi and alveoli, and the maximum concentration of total bacteria was associated with particles of diameter of 1.1-2.1 μm, size of bacteria. Clauss (2015) reported hospitals usually lack large-sized bacteria-laden particles because almost all hospitals have surface and air cleaning systems. The maximum concentration of *Staphylococcus* spp was associated with particles 7-12 μm in diameter indicating location on skin squamous, droplet and dust. Chaivisita *et al* (2018) observed bacteria-bearing particles of these dimensions have the potential of being deposited in the upper respiratory tract, a natural habitat of *Staphylococcus* spp including *S. aureus*, nasal colonization by the latter increases risk of nosocomial infection (Wertheim *et al*, 2004). In addition, once large-size bacteria-laden particles are emitted into air and settle on surface, they potentially constitute sources of bacterial transmission when standard and/or contact precautions are not rigorously followed (Zayas *et al*, 2012). The results of the current study emphasize the need of adequate ventilation and proper air and surface cleaning systems to control and mitigate bacterial aerosol and surface contamination. Mechanical ventilation systems are essential for diluting indoor air pollutants by removing contaminated indoor air and introducing clean outdoor air into closed air-conditioned rooms and buildings (Leung and Chan, 2006). Failure or malfunction of any component of the ventilation system will subject occupants to discomfort and exposure to airborne contaminants; consequently, an adequately installed ventilation system requires routine maintenance and monitoring to provide acceptable IAQ and minimize conditions favoring propagation of airborne pathogens (Sehulster *et al*, 2004).

In summary, indoor air quality assessment reveals high concentration of total bacteria and persistence of *Staphylococcus* spp including *S. aureus* in waiting rooms of five outpatient departments in a hospital in Pathum Thani Province, indicative of poor ventilation system, overcrowding and unsanitary quality of indoor air. Distribution of total bacteria and *Staphylococcus* spp with size ranges capable of being deposited in nasal cavity and lung poses health risks of acquiring nosocomial infections among patients and healthcare workers through contact or inhalation of the pathogens especially by vulnerable patients. This study was limited to a particular geographic area and should not be used as a general indicator of hospital indoor air quality across the country. The culture-based method employed in this study imposes limitations as to types and amounts of airborne bacteria that are detected. Further studies employing a combination of particle counting and PCR-based bacteria identification/quantification methods should be carried out to confirm findings from the current study. These types of indoor air quality monitoring programs should be routinely conducted not only in this particular hospital but in all hospitals where closed air conditioning systems are employed.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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