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Airborne and Surface-Bound Microbial Contamination in Two Intensive Care Units of a Medical Center in Central Taiwan

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ABSTRACT

Samples of airborne and surface-bound microbial contamination were taken in two intensive care units of a large-scale medical center. Microbial analyses included total bacterial and fungal loads, as well as the four bacterial species of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Among the 114 surface samples taken from seven designated surface locations of room and equipment for each patient, *P. aeruginosa* was the most frequently detected (19.3%) and most abundant (mean count: 4.18 cfu/plate) bacterium, whereas the respirator represented the most heavily contaminated surface location in both total pathogenic bacteria colony counts (272 cfu) and frequency of positive detection (38.2%). *P. aeruginosa* also represented the most frequently detected (39.1%, $n = 46$) and abundant (11.52 ± 17.16 cfu/m^3) bacterium in the air samples ($n = 46$), and was the only bacterium exhibiting a positive correlation of the mean counts between air and surface samples. The data analysis results further suggested a higher value of relative risk among the infected patients in the presence of the pathogens as compared to those in the absence of them, although the evidence of a of correlation for the individual bacterial species between the environmental samples and infected patients was inconclusive. This study also found that the mean airborne counts and the detection frequencies of these bacteria after patient visitation periods were higher than those before visitation, and that the installation of local air ionizers did not lead to any discernible differences in total bacterial and fungal concentrations.

Keywords: Indoor air quality; Nosocomial infection; Airborne pathogens; Intensive care units; Hospital environment.

INTRODUCTION

The indoor air quality of hospitals and medical centers has become a critical part of hospital management protocols. A number of studies have indicated that indoor air pollutants with chemical (Lu *et al.*, 2006; Chen *et al.*, 2009) and biological (Ortiz *et al.*, 2009; Wan *et al.*, 2011; Napoli *et al.*, 2012) natures pose potential hazards to patients, medical staffs, and visitors in hospitals. Sophisticated simulations through computational fluid dynamics for indoor air contamination and control design have also been developed (Panagopoulos *et al.*, 2011; Cao *et al.*, 2012). In particular, the importance of the airborne route of transmission in the epidemiology of healthcare-associated infection (HAI) has gained much attention in the past two decades, considering that patients susceptible to cross-transmission infection may result in significant increase in morbidity and mortality. For example, Li and Hou (2003) reported, in their study of 71 different cleanrooms in a hospital in Taiwan, that bacterial and fungal concentrations ranged from 1 to 423 and from 0 to 319 CFU/m^3, respectively, and that bacterial concentrations were higher than fungal ones. However, there were only weak relationships among class level, particle concentration and bioaerosol concentrations. Obbard and Fang (2003) conducted bioaerosol study in a hospital in Singapore, and showed that the occupant density was the key factor influencing the level of airborne bacteria, supported by the fact that the bacteria identified were representative of normal microflora of the skin, respiratory and gastro-intestinal tracts. Furthermore, Qudiesat *et al.* (2009) noted that, from their studies in two selected hospitals (a private and a public) in Jordan, the air quality in terms of biological

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contamination in the governmental hospital was worse than that of the private hospital in all units. In both hospitals, *Staphylococcus aureus*, *Micrococcus luteus* and coagulase-negative *Staphylococcus* ranked among the most common bacteria identified whereas fungal species *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Alternaria* spp. were isolated in both hospitals. These results are in parallel with those reported in a study for hospitals in India (Sudharsanam *et al.*, 2008). Nearly all studies showed that the level of biocontamination is strongly related to the occupant density as well as other factors such as construction (Mahieu *et al.*, 2000) and building age.

Hospitalized patients, especially those requiring extended treatments and intensive care, are at increased risk to exposure under bioaerosol contamination. A number of studies have shown that hospital-acquired infections are responsible for approximately 10% of the patients (Stamn *et al.*, 1977, Mayon-White *et al.*, 1988, Meers *et al.*, 1990). This risk of nosocomial infection is further escalated by the increasing prevalence of antibiotic-resistant pathogens such as methicillin (oxacillin)-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) among Gram-positive organisms, and multi-drug resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter* among Gram-negative organisms. Especially, *Acinetobacter baumannii* is becoming a major hospital-acquired infection issue because it is often multi-drug resistant (Wang *et al.*, 2003; Marais *et al.*, 2004; Pimentel *et al.*, 2005; Wilks *et al.*, 2006; Apisarnthanarak *et al.*, 2008). This contributes to the increase of morbidity and mortality. These studies all point out that active surveillance is necessary to reduce the risk of cross-contamination, and to possibly help identify the carriers.

Despite the increasing understanding of the hospital microbial hazards and control measures, studies on the surface-bound microbial contamination and their correlation with the airborne contamination are still very limited. The purposes of this study, therefore, were to examine the potential correlation between airborne and surface contamination in two intensive care units (ICUs), and whether patient infections were related to microbial contamination in the ICU. The study paid particular attention to the presence of drug-resistant species in MDR *A. baumannii* and MRSA, *P. aeruginosa*, as well as the common infectious bacteria in *Escherichia coli*. These bacteria are also among the most common pathogens of HAI in the ICUs of medical centers in Taiwan (Taiwan CDC, 2012). Additionally, the effects of air purifiers and patient visitation activities in the ICUs were also investigated in this study.

MATERIALS AND METHODS

Sampling Areas and Design

The areas of study included two separate ICUs which are located in the same building of a public, 1500-beded medical center in Central Taiwan. One of the ICUs, hereinafter referred to as ICU I, is a comprehensive ICU having a total area of 350 m^2 and housing 17 beds. The layout of ICU I (Fig. 1(a)) is designed with the nursing stations in the center of the open space and 15 beds on the perimeter, with tracked curtains to divide patient bed spaces. Air ventilation was provided by a centralized air conditioning, with a ceiling venting outlet above each of the beds. The other two beds were separately isolated in negative-pressure wards on the two opposite sides of the ICU. The air ventilation operated at a flow rate such that the air turnover rate was 5.2/hr. The ICU maintenance practices included daily cleaning, as well as sterilization by disinfectants and ultraviolet irradiation between patient exchanges.

Sampling in ICU I was carried out in two four-day cycles (August 30 through September 2, and October 3 through 6, 2011). Samples were taken from air (air sampling) and from surfaces (direct-contact sampling). For each bed space subjected to sampling, an air sample was taken near the head side of the patient bed, whereas the surface samples were collected from the following targets: medication trolley (top trey), bedrail (top side), cabinet (two locations on the frontal side), respirator (control knob), sputum suction apparatus (control knob), and physiological monitor (control button). The surface samples were taken only on the first day of each sampling cycle. Air samples from the odd number of beds were taken in the first and the third days in a cycle, while those from the even number of beds were collected in the second and the fourth day. At the time of the sampling period, all beds were occupied with patients in treatment, although patient exchanges occurred during the two sampling cycles.

ICU II is designated for patients receiving respiratory treatments. This ICU houses a total of 25 beds (Fig. 1(b)), including two beds in separate isolation wards on the two side of the entrance hallway. Only air samples were taken in ICU II as this part of the study was designed to examine the effects of air purifiers and patient visitation activities on the air quality. At the time of the study with two five-day cycles (cycle 1: August 29 through September 2; cycle 2: October 3 through 7, 2011), two identical sets of air ionizers were mounted on one side of the two pillars near the center of the open space. Specification of the air ionizer indicates that each set is effective within an area of 60 m^2. Air samples were taken near the head side of the four patient beds closest to the air ionizers, two directly facing each of the ionizers (approximately 1 m apart), and two on the back side of the pillars (approximately 3 m apart). Furthermore, to investigate the potential effect of visitation activity, air samples were collected at the same locations 30 min before ICU II was open for visitation. The same procedure was then repeated immediately after the visitation period was closed. These sampling activities were performed daily during the two study cycles.

Sampling Methods

Air samples were taken by using a 400-hole (0.25 mm), single-stage bioaerosol impactor (Standard BioStage, SKC Inc., PA, USA). Air samples were collected through a personal pump at a controlled flow rate of 28.3 L/min and a sampling duration of 2 min. The sampler was positioned between 1 and 1.5 m above ground, depending on the sampling purposes. The impactor was sterilized with 70%

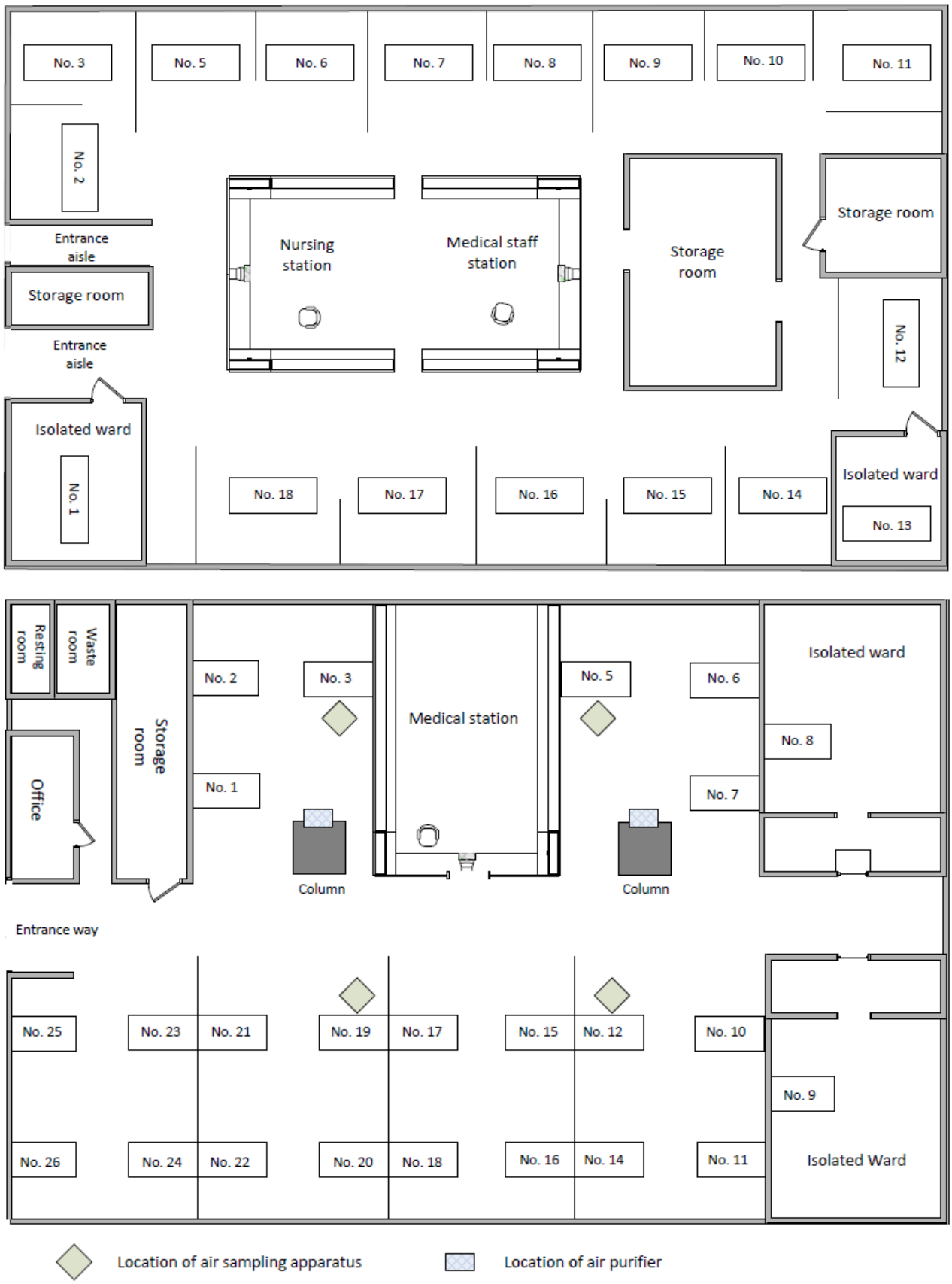

Fig. 1. Layouts of (a) ICU I: Air and surface samples from the odd number (○) and the even number (●) of beds were taken on alternating days between the sampling cycles; and (b) ICU II: air samples were taken at the locations as marked (◇).

ethanol prior to each sampling effort. The bioaerosols were collected on a 90-mm petri dish containing either tryptic soy agar (TSA) or malt extract agar (MEA) for culturing bacteria and fungi, respectively. Each of the used petri dishes was sealed with parafilm immediately after sampling, and was incubated at 35°C for 48 h at an offsite laboratory. To ensure sampling consistency, air samples were taken in ICU I and ICU II six consecutive times, with a 30-min interval between each sampling.

Direct-contact sampling was applied to collect surface-bound microorganisms. For each sampling effort, a new sterile cotton swab was lightly pressed on the target surface for 15 s. The swab was then immediately spread over a 90-mm petri dish for incubation. Media used for inoculation included CHROMagar Acinetobactor (CAA) and CHROMagar Orientation (CAO) (CHROMagar, Paris, France). CAO medium allows for the differentiation and identification of pathogens with characteristic appearances, including *S. aureus* (appeared in small golden colonies), *E. coli* (in dark pink), and *P. aeruginosa* (in opaque white), whereas CAA medium allows for the selective growth of MDR *A. baumannii*, exhibiting red appearance on the agar.

Microbial Isolation and Analytical Methods

Microbial growth after an incubation of 48 h was enumerated. In the direct-contact surface-bound samples, the colony counts were that directly read from the plate based on the number of colony forming unit (cfu/plate), whereas those for the air samples were corrected by using the positive-hole conversive table (Macher, 1989) and calculated as concentration unit in air (cfu/m^3).

All data was handled by Microsoft Excel and analyzed by JMP v. 9.0 (SAS Inc., USA). One-way analysis of variance (ANOVA) was applied to test the statistical significance of microbial distribution, as well as to evaluate the effects of air ionizers and visitation activities on the bioaerosol contamination. The correlations between airborne bioaerosol concentrations and surface-bound contamination in ICU I were analyzed using multivariate analysis by Spearman's index.

The relative risk (RR, with 95% confidence interval, CI), a ratio of the proportions of cases having a designated outcome in the exposed and the control groups, was adapted to partly evaluate the effect of indoor biocontamination on the infected patients. This method has been studied as an indicator of hospital contamination (Scaltriti *et al.*, 2007). ANOVA was also applied to test whether patient infection was statistically significant with indoor biocontamination.

RESULTS

Sampling Consistency Study

Prior to the scheduled sampling activities, the method consistency and representativeness were assessed by taking six consecutive air samples at the identical location in ICU I (near bed 17) and ICU II (near bed 12), each separated with a 30-min interval. There was no cleaning event in between sampling to ensure data integrity. For ICU I, the total bacterial colonies formed on TSA ranged from 15 to 30 cfu/plate, with a mean (± standard deviation) count of 22.2 ± 5.6 cfu/plate and a corresponding mean airborne concentration of 391.6 ± 99.0 cfu/m^3. In comparison, the number of total fungal colonies grown on MEA ranged between 0 and 3 cfu/plate, resulting in a mean concentration of 11.8 ± 21.4 cfu/m^3. Additionally, the numbers of colonies grown on CAA (4 to 6 cfu) and CAO (0 to 2 cfu) medium were also consistent throughout the test.

For the air samples taken ICU II, the mean bacteria concentration enumerated from the TSA medium was 356.4 ± 17.3 cfu/m^3, which was similar to the mean bacterial concentration obtained in ICU I. The mean fungal concentration (58.8 ± 34.6 cfu/m^3), however, was markedly higher than that from ICU I, as were the colony counts grown on CAA (3 to 12 cfu) and CAO (53 to 141 cfu). The total bacterial and total fungal concentrations in ICU I and II were below the recommended limits set by Taiwan Environmental Protection Administration (500 cfu/m^3 for bacteria, 1000 cfu/m^3 for fungi) (2011) and the mandatory limits set by the Korean Ministry of Environment (800 cfu/m^3 for bacteria) (2007) for medical-care facilities.

Airborne and Surface-Bound Microbial Concentrations in ICU I

Table 1 shows the mean plate counts and the corresponding standard deviation of the four targeted pathogens taken from the various surface and air samples, as well as the frequency of positive detection of these pathogens among these samples. A total of 114 surface samples were available, including 12 samples for the respirator sites and 17 samples for each of the other six surface sites. The mean count and the frequency of detection of these bacteria from the air samples were also presented in the table. Among the four bacteria studied, *P. aeruginosa* was the most frequently detected (19.3%) and the most abundant (a mean count of 4.18 cfu/plate). All surface sites experienced positive detection of *P. aeruginosa*, with one of the cabinet site and the sputum extractor knob being the most heavily contaminated sites. At the time of the study, the sputum sample analysis showed that seven of the 17 patients (42%) had been infected by *P. aeruginosa*, rendering the contamination in the medical equipment surfaces with which the medical staff most frequently contacted probable source of colonization and even spreading. Additionally, among the seven surface sites sampled, the respirator represented the most heavily contaminated site with respect to both total bacterial colony counts (272 cfu) and frequency of positive detection (38.2%).

Airborne Microbial Concentrations in ICU II

Table 2 reports the airborne microbial concentrations in ICU II, covering the data collected from two separate five-day sampling runs. Bacteria were presence in all air samples, whereas fungi growths were observed in 85% of the samples before visitation and 92.5% afterward. The mean bacterial counts were nearly identical before and after visitation, whereas the mean fungal count was less than that after visitation. Both bacterial and fungal counts were not significantly different before and after visitation.

Table 1. Quantification of detected from various surfaces and air in ICU I (units: cfu/plate).

Surface type \ Bacteria	*A. baumannii*	*S. aureus*	*E. coli*	*P. aeruginosa*
Bedrail	1.47 ± 5.56	ND	ND	5.12 ± 19.33
Sputum extractor knob	ND	0.65 ± 2.67	ND	7.47 ± 27.99
Cabinet 1	0.06 ± 0.24	ND	0.12 ± 0.48	10.06 ± 40.96
Cabinet 2	0.18 ± 0.73	ND	0.41 ± 1.46	0.12 ± 0.33
Medication trolley	1.29 ± 4.85	ND	1.59 ± 5.10	2.24 ± 6.14
Physiological monitor	ND	ND	ND	0.18 ± 0.73
Respirator knob	0.42 ± 1.16	14.67 ± 14.81	3.58 ± 8.70	4.00 ± 4.86
Detection frequency (%)	7.89	9.65	11.40	19.30
Air †	0.15 ± 0.42 (2.64 ± 7.39)	0.20 ± 0.68 (3.52 ± 12.0)	0.20 ± 0.50 (3.52 ± 8.80)	0.65 ± 0.97 (11.44 ± 17.07)
Detection frequency (%)	13.04	10.87	15.22	39.13

† The airborne bacterial quantification is expressed in both "cfu/plate" for numerical comparison with those obtained from the surfaces, and "cfu/m^3" (in parenthesis) for the convention of airborne concentration unit.
Note: 1. ND = not detected; 2. Sample size: n = 17 for each of the following surfaces: Bedrail, sputum extractor knob, cabinet 1, cabinet 2, medication trolley, physiological monitor; n = 12 for the respirator knob; n = 46 for air.

Table 2. Microbial concentration in ICU II air samples before and after visitation activities.

	Total bacteria			Total fungi			*A. baumannii*		
	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)
Before (n = 40)	483.2 ± 278.4	6, 70	100	106.9 ± 126.1	0, 26	85.0	2.65 ± 10.24	0, 3	7.5
After (n=40)	484.5 ± 249.8	7, 60	100	79.5 ± 84.0	0, 21	92.5	3.53 ± 9.96	0, 2	12.5
Significance (p-value)	0.49			0.87			0.34		

	S. aureus			*E. coli*			*P. aeruginosa*		
	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)
Before (n = 40)	0.88 ± 3.90	0, 1	5.0	4.42 ± 11.14	0, 3	17.5	11.48 ± 16.28	0, 3	42.5
After (n = 40)	4.42 ± 11.84	0, 3	15.0	6.63 ± 11.09	0, 2	30.0	10.60 ± 17.80	0, 5	37.5
Significance (p-value)	0.029*			0.18			0.59		

Among the four studied bacteria, *P. aeruginosa* was the most abundant and frequently detected species, followed in the order by *E. coli*, *S. aureus*, and *A. baumannii*. The extent and the frequency of colonization of the four bacteria in ICU II were generally consistent with those observed in ICU I. In most cases, the mean counts and the detection frequencies of these bacteria after visitation were higher than those before visitation, though statistically only the count of *S. aureus* was significantly different.

DISCUSSION

Airborne and Surface-Bound Bacteria Concentrations (ICU I)

As shown in Table 1, *S. aureus* represented the most abundant (14.67 cfu/plate) bacteria found on the respirator surfaces. This value was much greater than those reported by Perdelli *et al.* (2008) (1.8 cfu/plate) and Kerr *et al.* (2006) (0–57 cfu, n = 30), but appeared to be an isolated case considering that the frequency of positive detection and the mean count were 9.65% and 1.64 cfu/plate, values which were less than those of *P. aeruginosa* and not markedly different than the others. Nonetheless, this result reflects the strong possibility to spread the bacteria though indirect pathways such as handling contaminated objects by the medical staff. Growth of colonies with appearances different from the characteristic colors exhibited by the three target bacteria were also observed on the CAO medium. Two of the colonies having the highest frequency of appearance were isolated and subjected to additional

identification using GEN III Biolog method. The identification results showed that these colonies belonged to *S. capitis subsp. ureolyticus* (similarity 0.970), strains typically found from skin from head area (Bannerman and Kloos, 1997), and *S. pasteuri* (0.938), a possible bacteraemia as recently reported (Savini *et al.*, 2009).

Table 1 also shows the mean count and the positive detection frequency of the bacteria determined from the air samples, which were taken on the head side of each bed in ICU I. *P. aeruginosa* represented the most frequently detected (39.1%, $n = 46$) and abundant (0.65 ± 0.97 cfu/plate, or 11.52 ± 17.16 cfu/m^3) species. This result corresponded well with the finding from the surface sites. Furthermore, the order of positive detection frequency for the four bacteria from air samples coincided with those from the surface samples as well as with the percentage of patients infected with the corresponding bacteria (42% for *P. aeruginosa* and *E. coli*, 35% for *S. aureus*, and 33% for *A. baumannii*).

Further multivariate analysis using Spearman's index for correlation, as summarized in Table 3, showed that only *P. aeruginosa* had a significant ($p < 0.05$) but weak positive correlation of the mean counts between air samples and surface samples. No other microbial counts demonstrated any significant differences. It had been hypothesized that the airborne bioaerosols would eventually deposit onto the surrounding surfaces, and that the number correlation would form a positive correlation. Although the scheduled surface cleaning procedure performed in the ICU prevented any conclusive result from being obtained in such studies, the abundance of *P. aeruginosa* and its correlation in both air and surface samples should be alarmed, as its resistance to disinfectants has been previously reported (Russell, 1998).

Correlation between Bacterial Contamination with Infected Patients (ICU I)

During the studied period, the medical records concerning patients' infection screening results (sputum, blood, blood from catheter, pus, urine) indicated that one of the patients was simultaneously infected with all four bacteria, one was infected with three bacteria (*A. baumannii*, *E. coli*, and *P. aeruginosa*), two were infected with two bacteria, and thirteen were infected with one of the bacteria. The relative risk of the patients having been infected in the presence of the bacteria detected in ICU I (RR, 1.51; 95% CI, 0.91 to 2.51) was notably higher than the infected patients in the absence of the bacteria (RR, 0.82; 95% CI, 0.65 to 1.04). Also, the RR of the patients uninfected in the absence of the bacteria (RR, 1.21; 95% CI, 0.95 to 1.53) was higher than those uninfected in the presence of the bacteria (RR, 0.65; 95% CI, 0.39 to 1.09). We were therefore further interested in examining whether the distribution of the airborne and surface microbial concentration was spatially related to the patients' infection.

Table 4 shows the statistical results pertaining to the mean counts of the each bacterium determined from the collective samples (i.e., combining air and surface samples) surrounding the beds occupied with infected or uninfected patients specific to the bacterium. The results showed that, in cycle 1, the collective counts of *A. baumannii* was significantly ($p < 0.05$) greater for beds with infected

Table 3. Correlation of bacterial contamination between air and surface samples in ICU I (units: cfu/plate).

	A. baumannii	*S. aureus*	*E. coli*	*P. Aeruginosa*	Total bacteria	Total fungi
Surface count ($n = 114$)	0.49 ± 2.88	0.47 ± 3.09	1.86 ± 6.72	4.18 ± 20.53	53.98 ± 33.32	3.71 ± 2.87
Airborne count† ($n = 68$)	0.12 ± 0.37 (2.11 ± 6.51)	0.18 ± 0.46 (3.17 ± 8.10)	0.37 ± 0.75 (6.51 ± 13.20)	0.60 ± 0.88 (10.56 ± 15.49)	35.84 ± 39.69 (630.8 ± 698.5)	3.31 ± 5.76 (58.25 ± 101.4)
Correl. Coeff. (r^2)	–0.04	–0.49	0.01	0.08	–0.06	0.20
Significance (p-value)	0.53	0.40	0.47	0.0091*	0.42	0.94

n represents the number of samples.
† The corresponding airborne concentrations (cfu/m^3) are expressed in parenthesis.
*$p < 0.05$

Table 4. The mean and deviation counts of the bacteria for the infected and uninfected patients in ICU I (units: cfu/plate).

	Condition	*A. baumannii*	*E. coli*	*S. aureus*	*P. aeruginosa*
Cycle 1	Infected	0.80 ± 3.75	0.15 ± 0.59	0.17 ± 1.36	3.35 ± 17.31
	uninfected	0.06 ± 0.37	0.39 ± 2.58	0.00 ± 0.00	3.24 ± 0.20
	Significance (p-value)	0.0192*	0.2639	0.2960	0.9008
Cycle 2	Infected	0.03 ± 0.18	4.00 ± 7.88	1.73 ± 5.60	1.63 ± 2.77
	uninfected	0.44 ± 1.03	4.94 ± 12.57	0.19 ± 0.40	1.25 ± 3.21
	Significance (p-value)	0.0249*	0.1648	0.0342*	0.2184

Sample size: Cycle 1, total number of samples for infected patients (n_{inf}) = 65, total number of samples for uninfected patients (n_{uninf}) = 71; Cycle 2, $n_{inf} = 30$, $n_{uninf} = 16$.
*$p < 0.05$

patients than those with uninfected patients, but the reverse was true in cycle 2. Additionally, the *S. aureus* count for the beds with infected patients was also significantly greater than those with uninfected patients in cycle 2. The contradicting results for *A. baumannii* likely stem from the single surface-source (i.e., respirator) measurement in cycle 2 for some of the beds, and therefore the representativeness of the result is questionable. In reality, given that the sample size for the infected patients to each specific bacterium was small, combined with the frequent patient change-over (a patient normally stays in ICU I for 3 to 7 days) and the routine cleaning events, undisturbed study concerning bacterial spreading spatially relative to infected patients is difficult to perform. Substantially larger sample size and duration must be collected to obtain a more conclusive result.

Effects of Patient Visitation Activities and Local Air Purifiers

As reported in Table 2, the mean airborne bacterial counts in ICU II were nearly identical before and after visitation, whereas the mean fungal count was less than that after visitation. However, it is noteworthy that the microbial profiles between the two sampling runs were very different. For example, the airborne bacterial (334.8 ± 186.5 cfu/m^3) and fungal (61.8 ± 104.8 cfu/m^3) concentrations in the second run were noticeably less than those in the first run (631.6 ± 279.1 cfu/m^3 and 151.9 ± 131.8 cfu/m^3, respectively), suggesting that the air quality was generally better in the latter run. Conversely, the bacterial concentrations, except for *A. baumannii*, obtained in the second sampling run (*A. aureus*: 8.83 ± 14.61 cfu/m^3, *E. coli*: 1.77 ± 5.44 cfu/m^3, *P. aeruginosa*: 12.37 ± 15.27 cfu/m^3) were markedly higher than those in the first run (0.0, 0.0, and 10.60 ± 17.57 cfu/m^3, respectively), as were the detection frequencies. In additional to the turnover of patients in the ICU, there was a section-wide cleaning event in between the sampling runs. The reduction of bacterial and fungal loads and the general improvement of the indoor air quality can therefore be reasonably attributed to the cleaning.

The increased airborne bacterial concentration even after the cleaning implies that the occurrence of these bacterial contaminations was likely due to isolated events or conditions rather than the scheduled cleaning activity. This was also supported by the observation that only the *A. baumannii*, *S. aureus*, and *E. coli* concentrations increased (but none were statistically significant) after visitation for the first sampling run, whereas the total bacteria, *S. aureus*, *E. coli*, and *P. aeruginosa* concentrations increased after visitation in the second sampling run. Since *S. aureus* is frequently found as part of the normal skin flora on the skin and nasal passages, its increase after visitation can be justified as being transported through visitors. However, the distinctly patterns of microbial concentration change between the two sampling runs render the effect of visitation inconclusive, as other transportation routes may also play critical roles in the airborne concentration increases of these bacteria.

Tang *et al.* (2009) reported their findings concerning the effect of patient visitation on the indoor air quality in a small ICU. They concluded that the CO_2, coarse particle (i.e, particulate size greater than 10 μm) and fungal concentrations were significantly greater after patient visitation, and that the coarse particle concentration showed strong correlation with the number of visitors in the ICU, whereas the bacterial concentration did not differ significantly. In this study, we did not observe increase in either CO_2 or fungal concentrations, but did observe significant bacterial concentration increase in the second sampling run. Again, the inconsistent findings between the studies indicate that the adverse effects of patient visitation contributing to airborne contamination is difficult to identify, but sufficient ventilation and stringent enforcement for the visitors to wear medical gowns and masks are necessary to reduce risks of direct human contact through skin and respiration.

Air ventilation approaches such as maintaining sufficient air exchange rates (ANSI/ASHRAE, 2003), coupled with air filtering has become a fundamental technique to ensure air quality in hospital settings (Stacey and Humphreys, 2002; Anttila *et al.*, 2009). To control the exogenously-acquired healthcare-associated infection and transmission, most hospitals use isolation room and pressurization control (e.g., negative pressure room) for areas highly sensitive to infection, such as operating theater and ICUs. Local air purifiers involving filtration units and chemical oxidizers (e.g., ozone and ionized air) have also been installed in various clinical units to control microbial contamination, normally in areas with high occupant density or inadequate ventilations. As depicted in Fig. 1(b) for this study, as pair of air ionizers were equipped in ICU II, and Table 5 reports the airborne microbial concentrations for samples collected at the face-side and the back-side of the air ionizers. We had previously hypothesized that the air quality near the beds located on the face-side of the purifiers would be improved than elsewhere, but the results showed no discernible differences of total bacterial and fungal concentrations for the two sampling positions. Also, except for *A. baumannii*, the mean concentrations and detection frequencies for the bacteria were actually higher on the face-side than those on the back-side. None of the differences were statistically significant.

In a study by Kerr *et al.* (2006) that compares the presence of MRSA and *Acinetobacter* spp. in the presence and absence of a negative air ionization in an ICU, they reported there was a significant reduction of the incidence of nosocomial *Acinetobacter* spp. infection or colonization after the air ionizers were operational, but that of MRSA remained unchanged. An increase of *Acinetobactor* spp. in the air as well as on the inanimate surfaces was also reported in that study. Other successful applications of air ionizers have also been reported for particle control in closed environment (Shiue *et al.*, 2011; Sawant *et al.*, 2012). In this study, though the efficient air ventilation that homogenize the air quality in the ICU may explain the lack of noticeable microbial concentration differences on the two sides of the air purifiers, one may still argue that the local air purifiers do not work well in open-space ICU. Additionally, studies using air filtration systems could produce different results than Kerr's and our study, which both used non-filter air purifiers. For example, Mahieu *et al.* (2000) reported that

Table 5. Microbial concentration in ICU II air samples for sites on the face and back sides of air purifiers.

	Total bacteria			Total fungi			*A. baumannii*		
	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)
Face side (n = 40)	483.2 ± 268.1	9, 70	100	94.9 ± 106.1	0, 23	85.0	2.65 ± 9.43	0, 2	7.5
Back side (n = 40)	484.5 ± 260.7	6, 57	100	91.4 ± 109.9	0, 26	92.5	3.53 ± 10.74	0, 3	12.5
Significance (p-value)	0.49			0.43			0.33		

	S. aureus			*E. coli*			*P. aeruginosa*		
	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)	$\bar{c} \pm \delta$ (cfu/m^3)	min, max (cfu/plate)	Detection frequency (%)
Face side (n = 40)	2.65 ± 10.24	0, 2	12.5	6.18 ± 12.36	0, 3	25.0	13.25 ± 20.30	0, 5	42.5
Back side (n = 40)	2.65 ± 7.54	0, 3	7.5	4.86 ± 9.76	0, 2	22.5	8.83 ± 12.65	0, 2	37.5
Significance (p-value)	0.50			0.29			0.12		

the application of an air filter unit (consisting of a prefilter, an activated carbon filter, and a high-efficiency particulate air (HEPA) filter) in a neonatal ICU effectively reduced the airborne concentration of *Aspergillus* spp. The study by Araujo *et al.* (2008) also indicated that wards equipped with HEPA filters at positive air flow yielded lower fungal levels, particularly for penicillia. The paradoxical findings between these studies entail further verification whether localized air purifiers may be effective in controlling the airborne pathogens in ICUs.

CONCLUSIONS

We have investigated the extent of microbiological contamination in two independent ICUs in a public medical center in Taiwan. The ICUs, one of which was a medical ICU (ICU I) and the other a respiratory ICU (ICU II), are both subjected to the same cleaning and disinfection procedures. The extents of total bacterial and fungal contamination in air were similar in the two ICUs in the first sampling cycle, with *P. aeruginosa* as the most abundant and frequently detected bacterium. Also, we observed in ICU I that there existed a limited trend between the surface and airborne bacterial contamination, although the results of the bacterial multivariate analysis showed that only *P. aeruginosa* had a significant ($p < 0.05$) but weak positive correlation of the mean counts between air samples and surface samples. Based on the observed persistent of the bacteria, particularly of *P. aeruginosa*, in the environment (air and surfaces), we suggest that the disinfection-resistant potential of the pathogens should be periodically tested. We have also noted a definitive higher value of relative risk among the pathogenic infected patients in the presence of the bacteria as compared to those in the absence of the bacteria. However, studies concerning the correlation of individual bacteria between the environmental samples and infected patients were inconclusive, as only the collective counts of *A. baumannii* in the first sampling cycle and of *S. aureus* in the second cycle, were significantly ($p < 0.05$) greater for rooms with infected patients than those with uninfected patients.

We also noted that the airborne bacterial and fungal concentrations in ICU II were not significantly different before and after patient visitation activities. However, the bacterial profiles were markedly different between the two sampling cycles implied that monitoring of total airborne bacterial concentration may not be sufficient for sensitive areas, as it does not reflect the extent of contamination of the bacteria of specific concern. Using non-filter air purifiers were not effective to control the microbial contamination.

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