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Particle and bioaerosol characteristics in a paediatric intensive care unit

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ABSTRACT

The paediatric intensive care unit (PICU) provides care to critically ill neonates, infants and children. These patients are vulnerable and susceptible to the environment surrounding them, yet there is little information available on indoor air quality and factors affecting it within a PICU. To address this gap in knowledge we conducted continuous indoor and outdoor airborne particle concentration measurements over a two-week period at the Royal Children's Hospital PICU in Brisbane, Australia, and we also collected 82 bioaerosol samples to test for the presence of bacterial and viral pathogens. Our results showed that both 24-hour average indoor particle mass (PM₁₀) (0.6–2.2 µg m⁻³, median: 0.9 µg m⁻³) and submicrometer particle number (PN) (0.1–2.8 × 10³ p cm⁻³, median: 0.67 × 10³ p cm⁻³) concentrations were significantly lower (p < 0.01) than the outdoor concentrations (6.7–10.2 µg m⁻³, median: 8.0 µg m⁻³ for PM₁₀ and 12.1–22.2 × 10³ p cm⁻³, median: 16.4 × 10³ p cm⁻³ for PN). In general, we found that indoor particle concentrations in the PICU were mainly affected by indoor particle sources, with outdoor particles providing a negligible background. We identified strong indoor particle sources in the PICU, which occasionally increased indoor PN and PM₁₀ concentrations from 0.1 × 10³ to 100 × 10³ p cm⁻³, and from 2 µg m⁻³ to 70 µg m⁻³, respectively. The most substantial indoor particle sources were nebulization therapy, tracheal suction and cleaning activities. The average PM₁₀ and PN emission rates of nebulization therapy ranged from 1.29 to 7.41 mg min⁻¹ and from 1.20 to 3.96 p min⁻¹ × 10¹¹, respectively. Based on multipoint measurement data, it was found that particles generated at each location could be quickly transported to other locations, even when originating from isolated single-bed rooms. The most commonly isolated bacterial genera from both primary and broth cultures were skin commensals while viruses were rarely identified. Based on the findings from the study, we developed a set of practical recommendations for PICU design, as well as for medical and cleaning staff to mitigate aerosol generation and transmission to minimize infection risk to PICU patients.

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1. Introduction

Maintaining high levels of indoor air quality in hospitals is important for protecting both staff and patients (Jung et al., 2015). The transmission of infectious diseases within hospitals poses a significant health risk and is of significant clinical concern (Cornejo-Juarez et al., 2015). Despite the implementation of infection control policies, transmission of infections within hospitals continues to occur (Siegel et al., 2007; Assiri et al., 2013). These transmissions could be patient-to-patient, visitor-to-patient, patient-to-health care worker and health care worker-to-patient.

The available evidence about the airborne spread of infection is limited, although transmission has been described for tuberculosis, measles, chickenpox and pertussis and multidrug-resistant non-tuberculous mycobacterium (Leclair et al., 1980; Bloch et al., 1985; Dharmadhikari et al., 2012; Warfel et al., 2012; Bryant et al., 2016). Other studies have suggested opportunistic spread of influenza virus and respiratory syncytial virus (RSV) via an airborne route (Roy and Milton, 2004; Killingley and Nguyen-Van-Tam, 2013; Luongo et al., 2016; Kulkarni et al., 2016). Further, Zingg et al. (2015) and Arefian et al. (2016) pointed out to the possibility of poor indoor air quality resulting in increased morbidity for health care workers, extended patient hospitalization as a result of hospital-acquired illnesses and also to increased economic costs.

Typically, hospitalized patients are triaged according to illness and level of care required, with the most critical patients admitted to an intensive care unit (ICU). Critically ill neonates, infants and children are cared for separately within the paediatric intensive care unit (PICU) (Bennett and Bion, 1999). A number of factors have been associated with increased risk of infection transmission among hospitalized patients. These include the increased number of airborne particles generated by activities such as suction, nebulization and cleaning especially at times when patient admissions are highest and when seasonal viruses are at their peak (Eidelman et al., 2009; Leung et al., 2014; WHO, 2014). Parents, guardians, visitors and hospital staff who are shedding viruses also increase the risk of hospital-acquired infection and contribute to increased particle concentration (Voinin et al., 2009). The quality of the air supply within the PICU environment is also important, as airborne pollutant exposure may affect the health of staff and patients. Currently, only one study has been conducted to investigate indoor bioaerosol characteristics in the PICU (Li and Hou, 2003).

The aim of this study was to improve our knowledge of indoor airborne particle and bioaerosol characteristics in a PICU environment. The objectives were: (1) to measure indoor and outdoor particle number (PN) and particle mass (PM_{10}) concentrations for two weeks continuously and simultaneously; (2) to identify particle sources and estimate particle emission rates; (3) to assess the filtration efficiency of the building ventilation system filters; (4) to analyze the spatial distribution and variation of particle concentrations; and (5) to collect bioaerosol samples and identify examples of airborne bacteria or viruses.

The main reasons for measuring particle concentrations and particle sizes are to understand the airborne particle size distribution and the type of airborne aerosol particle; identify the possible source of the particles; and assess their effect on human health.

It is, however, important to note that this study did not seek to investigate or establish whether microbial transmission occurred between patients, or between patients and visitors.

2. Experimental methods and data processing

2.1. The sampling site

This study was conducted in a PICU at the Royal Children's Hospital, Brisbane, Australia. The floorplan of the PICU is shown in Fig. 1. The

PICU consisted of 8 beds, of which Bed 1 and Bed 2 were located in separate rooms, and the others were situated in a common area. The size of this PICU is approximately 231 m². A nurses' station was located in the centre of the PICU. A mechanical air-handling unit (AHU) with two-stage filtration provided a constant flow of supply air to the PICU. The first stage filters (dry media filters) functioned in the same way as those in a typical mechanical ventilation system, with a filtration efficiency of 45–65%, while the second stage filters were high efficiency particulate air filters (HEPA) (Econocell Filters, Australia). There were four return air outlets, which were located above Beds 5 and 6, between Beds 5 and 6, and in the visiting room. In general, the first and second stage (HEPA) filters of the ventilation system were changed after approximately 1–2 years and 2–3 years, respectively.

2.2. Instrumentation and data collection

2.2.1. Particle number (PN)

Indoor supermicrometer $PN_{0.5-20\text{ }\mu\text{m}}$ concentration and size distribution (from 0.5 to 20 μm) were measured using a TSI Model 3312A Ultraviolet Aerodynamic Particle Sizer (UVAPS, TSI Incorporated, Shoreview, MN, USA), with a time resolution of 20 s. Indoor supermicrometer particle number $PN_{0.3-25\text{ }\mu\text{m}}$ concentration and size distribution (from 0.3 to 25 μm) were measured using a TSI AeroTrak Handheld Particle Counter 9306 (OPC, TSI Incorporated). The two instruments provided information on indoor spatial variations of supermicrometer particle concentration. Indoor and outdoor total submicrometer $PN_{0.005-3\text{ }\mu\text{m}}$ concentrations were measured using three TSI Model 3787 Condensation Particle Counters (CPC, TSI Incorporated) in the size range from 0.005 to 3 μm , and with a time resolution of 10 s (indoor) or 20 s (outdoor). Two of the instruments were operated indoors and one sampled outdoor air and was located next to the AHU outdoor air intake. One TSI Model 3007 CPC and one TSI P-Track were used for short-term (daytime) indoor total submicrometer $PN_{0.01-1\text{ }\mu\text{m}}$ and $PN_{0.02-1\text{ }\mu\text{m}}$ measurements, respectively.

2.2.2. Particle mass (PM_{10}) concentration (mass concentration of particles with an aerodynamic diameter smaller than 10 μm)

Indoor and outdoor PM_{10} concentrations were simultaneously measured by a TSI DustTrak-DRX and a TSI DustTrak II, respectively. Given that this work focused on an area with multiple potential sources of particles, including sources of particles larger than 2.5 μm (e.g. human-generated aerosols, resuspension etc.), it was appropriate to measure particle mass concentrations in terms of PM_{10} , rather than $PM_{2.5}$.

2.2.3. Indoor bioaerosols

Indoor bioaerosol samples were collected using a Coriolis μ Air Sampler (Bertin Technologies, France). Each sample, consisting of 3 m³ of air collected at 0.3 m³ per minute, was drawn into a conical sterile tube containing 15 ml of saline solution (0.85% NaCl). Normal saline or 0.9% sodium chloride is not mandatory, but is the preferred solution for bacterial manipulations as it is an isotonic solution and therefore the osmotic pressure of the bacterial cells is maintained. Saline is also a suitable medium to retain viral integrity prior to nucleic acid extraction for PCR. Due to the high flow rate used for sampling, it was possible that some of the liquid became aerosolized during sample collection. To contain these particles and prevent them from being released into the indoor air of the PICU, the instrument was placed in a plastic bag, with a HEPA filter at its outlet to remove any particles generated by the Coriolis μ Air Sampler.

Time series of indoor and outdoor CO₂, temperature and relative humidity were measured by a TSI Q-Traks (TSI 7545).

2.2.4. Other parameters

Supply and return air velocities were monitored using a TSI Model 8330 anemometer (VelociCalc) and a TSI Model 8705 DP-Calc

Fig. 1. PICU plan (B: Bed space; there are moveable curtains between bed spaces 3–8).

Micromanometer, respectively.

All instruments were tested and calibrated in the laboratory prior to the PICU measurements, and co-located comparative tests for all particle instruments were conducted simultaneously during the last day of the measurements.

Data on local outdoor PM_{10} concentrations and meteorological parameters were collected from a nearby regulatory air quality monitoring station (5 km away), and an Australian Bureau of Meteorology weather station located in Brisbane (10 km away), respectively.

2.2.5. Nasal swabs

De-identified nasal swabs were collected twice (PICU-inbound and PICU-outbound) between 8:00 am and 5:00 pm from each consenting adult who visited the PICU on a single day. One nostril was swabbed with a sterile swab, which was placed in a sterile saline solution, then the vial capped and sent to the laboratory for storage and batch processing. In total, 152 swabs were collected.

The detection rate for the primary cultures is 46% and 8% pathogens.

2.3. Study design

This study was conducted in the PICU from 4 to 16 August 2014, which was during the peak influenza season in Brisbane, and when hospital admissions were high.

Continuous indoor measurements were conducted using two sets of instruments. One set (including the UVAPS, CPC 3787 and DustTrak) was located in the center of the PICU throughout the entire measurement campaign. To determine indoor particle transport and dispersion, the second set of instruments (including the OPC and CPC 3787) was moved among a variety of locations throughout the entire measurement campaign, and the measurements at each location were conducted for at least 24 h. Additionally, the hand-held TSI P-Track and/or Model 3007 CPC were added for short-term (daytime 8 h) indoor total

submicrometer PN measurements at several locations within the ward. Thus, there were three or four places where indoor total submicrometer PN measurements took place simultaneously.

Continuous outdoor measurements were conducted using the third set of instruments including a CPC 3787 and DustTrak, which were located in the plant room throughout the entire measurement period.

Supply air PN concentrations were measured for approximately 15 min at each of the air outlets (total of 15) of the PICU by the P-Track. The tests were conducted at five points: one in the center (C1, C2, C3, C4 and C5), and one along each of the four sides of the outlet (S1 to S4) of each outlet. The tests were conducted once a day on several different days of the measurement campaign. Overnight supply air PN concentration monitoring was conducted using a CPC 3787 at two outlets, on two occasions throughout the monitoring period.

Supply air velocities were measured at each outlet using the VelociCalc. The measurement method was the same as for supply air PN concentrations. Overnight supply velocities were continuously monitored by the DP-Calc at two outlets for > 24 h. The filtration efficiencies of the two-stage filters were tested by using two CPCs and two DustTraks.

In all cases, one bioaerosol sample was collected at the center of the PICU, followed by samples collected 1 m from each patient bed (when possible), and then one final sample from the center location. This sampling process was conducted twice a day during the daytime (8:00–17:00). One sample was collected in the morning and one was collected in the afternoon; thus two samples were collected daily from each patient bed space. Nasal swab samples were collected on one of the sampling days.

For each patient being cared for in the PICU during the study, de-identified clinical data were reviewed to confirm the presence or absence of a current respiratory infection. The number of patients and people in the PICU was recorded by observation every 2 h throughout each day (from 7:00 am to 6:00 pm). We paid particular attention to recording when and where aerosol-generating clinical activities, such as

nebulization therapy and airway suctioning, occurred. This study was approved by the Children's Health Services Queensland Human Research Ethics Committee (No. HREC/14/QRCH/147).

2.4. Analysis of biological samples

The biological samples were analyzed to characterize their bacterial and viral content.

2.4.1. Processing of indoor bioaerosol samples for bacterial isolation

Following collection of a bioaerosol sample using the Coriolis μ Air Sampler, the saline collection was processed using two methods (primary sampling and broth cultures) to isolate potential bacterial pathogens. Processing of the primary sample was as follows. A 0.1 ml aliquot of the neat saline collections was subcultured directly onto a blood agar plate (Thermo Scientific, Australia) and a MacConkey agar plate supplemented with crystal violet (Thermo Scientific, Australia) and incubated for a total of 72 h at 37 °C, under 5% CO₂ and 0% CO₂ atmospheres, respectively.

The broth culture was processed using a 1 ml aliquot of the sample that was centrifuged at 14,000 rpm for 2 min. The supernatant was discarded and the pellet was resuspended in 2 ml of Luria Bertani Broth (Oxoid Ltd., Australia) and incubated overnight in a 0% CO₂ atmosphere. Following the incubation period, 0.1 ml of the sample was subcultured onto a blood agar plate and a MacConkey agar plate supplemented with crystal violet and incubated for a total of 72 h under 5% CO₂ and 0% CO₂ atmospheres, respectively. All agar plates were reviewed daily for growth of bacterial pathogens.

2.4.2. Bacterial identification

Phenotypic tests, including morphology, gram stain, oxidase (Becton, Dickinson and Company) and Staphytest Plus (Oxoid Ltd., Australia) latex agglutination test were used to determine if the isolate would be considered a pathogen or a skin commensal. All presumptive pathogens were stored in 15% glycerol in a –80 °C freezer.

The presumptive bacterial pathogens were subcultured from the freezer onto blood agar plates prior to identification using the Vitek® MS Mass Spectrometry MALDI-TOF instrument (bioMérieux Pty Ltd., Australia).

2.4.3. Processing of indoor bioaerosol and nasal swab samples for viral screening

Total nucleic acids were extracted from 0.2 ml of the impacted Coriolis liquid and from 0.2 ml of each of 152 swabs after each swab was resuspended by vortexing in 1 ml of OptiMEM® I (Gibco, Australia) medium, followed by purification using High Pure Viral Nucleic Acid spin columns (Roche Diagnostics, Australia) according to the manufacturer's instructions. Primary specimens and purified nucleic acid extracts were stored at –70 °C. Extracts (2 μ l) were tested by RT-PCR (SensiFAST™ One-Step, Bioline, Australia) or PCR (MyTaq™ DNA Polymerase, Bioline, Australia), as appropriate for the viral genome. All swabs were assessed for a panel of respiratory viruses (influenza virus A and B, human adenoviruses, RSV, human metapneumovirus [HMPV], human parechoviruses and human rhinoviruses [HRVs]) using individual real-time RT-PCR as described previously by Chang et al. (2009), Arden et al. (2010) and Lu et al. (2008).

The rhinovirus assay was determined to have a dynamic range of 9 log₁₀ copies of in vitro transcribed HRV-16 genomic RNA and could reliably detect 4 copies per reaction volume (data not shown).

Additional real-time RT-PCR testing for parainfluenza virus (HPIV) 1–3 (Chang et al., 2009) and human coronaviruses (HCoV) (Mackay et al., 2012) was undertaken on the 60 nasal swabs. A housekeeping gene real-time RT-PCR assay targeting the human UBE2D2 gene was used on the nasal swab extracts to determine the presence of human cellular mRNA as a measure of successful swab sampling (Chiu et al., 2006).

All RT-PCRs consisted of a 20 min pre-cycling incubation at 45 °C and a 2 min pre-cycling incubation at 94 °C followed by 35 cycles of 94 °C/5 s, 60 °C/60 s. PCR cycling was preceded only by a 15-min incubation at 94 °C followed by 35 cycles of 94 °C/10 s, 60 °C/60 s.

2.5. Estimation of emission rates of nebulizer therapy activity

When particle dynamics such as condensation, evaporation and coagulation are negligible, the main factors influencing indoor particle concentrations are emissions from indoor sources, penetration of outdoor particles, particle loss due to deposition on indoor surfaces, and air exchange rates (Morawska and Salthammer, 2003). The equation which relates these parameters has been reported in our previous studies (He et al., 2004; Morawska et al., 2009; Quang et al., 2014):

$$\frac{dC_{in}}{dt} = P \cdot \alpha \cdot C_{out} + \frac{Q_s}{V} - (\alpha + k) \cdot C_{in} \quad (1)$$

where C_{in} and C_{out} are indoor and outdoor particle concentrations, respectively, P is the penetration efficiency, k is the deposition rate, α is the air exchange rate, Q_s is the indoor particle generation rate, t is time and V is the effective volume of the space. For mechanical ventilation conditions, Eq. (1) can be modified to:

$$\frac{dC_{in}}{dt} = \frac{F \cdot C_{supply}}{V} + \frac{Q_s}{V} - (\alpha + k) \cdot C_{in} \quad (2)$$

where F is supply air flow rate, and C_{supply} is supply air particle concentration. The average emission rate, Q_s , can therefore be estimated by using average parameters and the following equation:

$$\overline{Q_s} = V \left[\frac{C_{int} - C_{in0}}{\Delta T} + \frac{\overline{C_{in}}}{(\alpha + k)} - \frac{F \cdot C_{supply}}{V} \right] \quad (3)$$

where C_{int} and C_{in0} are the peak and initial indoor particle concentrations, respectively, $\overline{C_{in}}$ is the average indoor particle concentration; $(\alpha + k)$ is the average total removal rate (which can be calculated using average decay rate after the source ceased operating), $\overline{\alpha}$ is the average air exchange rate, and ΔT is time difference between the initial and peak concentrations.

2.6. Data analysis

Data from the online particle measurements were grouped according to their location and time period. The total 24 h average indoor and outdoor concentrations were calculated for each indoor location, and used to determine the indoor/outdoor (I/O) concentration ratios. All statistical analyses (t -tests) were conducted using SPSS for Windows version 10 (SPSS Inc.). A 5% level of significance (two-tailed) was used in all analyses.

3. Results

During the measurement period, the PICU operated as normal. The average number of people in the PICU was 13.4 with a maximum of 26 (12:00 pm) and a minimum of 5 (8:00 am) (see Supporting Information Fig. S1).

3.1. 24-hour average indoor and outdoor particle concentrations

The overall average 24 h indoor PM₁₀, PN_{0.005–3 μ m} and PN_{0.5–20 μ m} concentrations were $1.1 \pm 0.5 \mu\text{g m}^{-3}$, $0.93 \pm 0.94 \times 10^3 \text{ p cm}^{-3}$, and $0.13 \pm 0.09 \text{ p cm}^{-3}$, respectively. Daily 24 h average indoor and outdoor PM₁₀ and PN_{0.005–3 μ m} concentrations are presented in Fig. 2. Both the average indoor PM₁₀ ($0.6\text{--}2.2 \mu\text{g m}^{-3}$, median: $0.9 \mu\text{g m}^{-3}$) and PN_{0.005–3 μ m} ($0.1\text{--}2.8 \times 10^3 \text{ p cm}^{-3}$, median: $0.67 \times 10^3 \text{ p cm}^{-3}$) concentrations were significantly lower ($p < 0.01$) than the outdoor concentrations ($6.7\text{--}10.2 \mu\text{g m}^{-3}$, median: $8.0 \mu\text{g m}^{-3}$ for PM₁₀ and $12.1\text{--}22.2 \times 10^3 \text{ p cm}^{-3}$, median: $16.4 \times 10^3 \text{ p cm}^{-3}$ for

Fig. 2. 24-h average indoor and outdoor PM₁₀ (A) and PN (B) concentrations.

PN_{0.005–3 µm}). The daily 24 h average outdoor PM₁₀ concentrations measured at the hospital were comparable with those at the nearby monitoring station during the measurement period (Fig. S2). The I/O ratio ranged from 0.07 to 0.26, with a median value of 0.11 for PM₁₀, and from 0.01 to 0.19, with a median value of 0.04 for PN_{0.005–3 µm}, respectively. Therefore, the 24 h indoor concentrations were only about 11% and 4% of the 24 h outdoor PM₁₀ and PN_{0.005–3 µm} concentrations, respectively. It can also be seen from Fig. 2 that the 24 h indoor concentrations varied from day to day for both PM₁₀ and PN_{0.005–3 µm}, but particularly for PN_{0.005–3 µm}. Similar variation was seen in the 24 h average indoor supermicrometer PN_{0.5–20 µm} concentrations, with a range of 0.052 p cm⁻³ to 0.376 p cm⁻³ and a median of 0.095 p cm⁻³ (Fig. S3).

3.2. Relationship between indoor and outdoor concentrations

Based on daily 24 h average indoor and outdoor concentration data, the relationships between indoor and outdoor concentrations of both PM₁₀ and PN_{0.005–3 µm} were very weak ($R^2 = 0.08$), as were the relationships between diurnal variations of indoor and outdoor concentrations. This implies that indoor concentrations were mainly affected by indoor sources, and to a lesser extent by outdoor air. An example of the diurnal variation of indoor and outdoor concentrations is given in Fig. 3, which shows that both indoor PM₁₀ and PN_{0.005–3 µm} concentrations increased due to nebulization therapy, cleaning activities and operation of other indoor sources. In general, however, when there were no indoor particle sources operating, indoor particle

concentration, particularly of PN_{0.005–3 µm}, followed the trend of outdoor concentration.

3.3. Supply air particle concentrations

A summary of the average PN_{0.02–1 µm} concentrations of supply air measured at each outlet is presented in Fig. 4 (the location of each supply air outlet is shown in Fig. 1), showing that the concentrations varied significantly between outlets. All the results for the average PN_{0.02–1 µm} concentrations at each of the five points of the outlet supply air measurements are shown in Table 1. The highest average PN_{0.02–1 µm} concentration of supply air was found at the SR (store room) outlet, which was likely due to lack of a HEPA filter. The second highest concentration was found at the C1 (center 1) outlet. Notably, the PN_{0.02–1 µm} concentrations at S1 and S3 were about 20 times higher than those at C, S2 and S4, which indicates that there may have been leaks on side 1 and side 3 of the C1 supply air outlet. The overall average PN_{0.02–1 µm} concentration of the supply air ranged from 1.9 p cm⁻³ to 329 p cm⁻³, with a median value of 20.6 p cm⁻³ and average value of 50 ± 99 p cm⁻³ (excluding supply air PN_{0.02–1 µm} concentrations at SR).

An example time series of outdoor and supply air (at Bed 7 outlet) PN_{0.005–3 µm} concentrations measured by two CPC 3787s is shown in Fig. 5, highlighting that the concentrations followed the same pattern. However, the average PN concentration of the supply air (1 p cm⁻³) was about 0.008% of that in outdoor air (12.2×10^3 p cm⁻³). These results show that indoor particle concentrations in the PICU were

Fig. 3. Time series of indoor and outdoor PM₁₀ and PN_{0.005–3 µm} concentrations (11 August 2014).

Fig. 4. Supply air particle concentrations of supply air outlets in the PICU. Note: B#, Bed number; C#, Center number; SR, Store room. The lower and upper boundaries of the box specify the 25th and 75th percentiles, respectively. The line within the box indicates the median, and the whiskers above and below the box indicate the maximum and minimum, respectively.

Table 1

Summary of the average PN concentration (p cm^{-3}) in the supply air from the Center 1 outlet, at five different points surrounding the outlet.

	C	S1	S2	S3	S4
Average	12.8	788	33.3	750	16.0
S.D.	6.1	338	5.8	350	3.6
Min	6.0	500	30.0	350	13.0
Max	20.0	1150	40.0	1000	20.0
Median	12.5	750	30.0	900	15.0

Note: C, center; S, side, 1, 2, 3, 4 are clockwise four directions (90 degree clockwise rotation).

mainly affected by indoor particle sources, with outdoor particles providing a negligible background.

3.4. Indoor particle sources

Based on the time series of indoor and outdoor PN concentrations, in combination with the clinical activity records, the most significant indoor particle sources were found to be nebulization therapy and cleaning activities. In addition, it was seen that invasive tracheal

suctioning performed by nurses or medical staff might have been another important indoor particle source. It was not possible to link all the peaks in particle concentrations with the sources generating them, due to the lack of activity records, particularly during the night, or because they were simply not recognized or recorded. Fig. 6 shows an example of a time series of indoor and outdoor $\text{PN}_{0.005-3 \mu\text{m}}$ and indoor $\text{PN}_{0.5-20 \mu\text{m}}$ concentrations, as well as indoor and outdoor $\text{PN}_{0.005-3 \mu\text{m}}$ and PM_{10} concentrations in the PICU. From this figure, the effect of indoor sources on indoor particle concentrations can be seen, and in particular that there were many peaks in both supermicrometer and submicrometer particle concentrations, which suggests that there were different types of indoor particle sources. Some sources generated only supermicrometer particles (such as dusting, changing bed sheets, coughing), others generated only submicrometer particles (such as mopping the floor with detergent), while some produced both types of particles (such as nebulization therapy). A summary of the daily maximum peak concentrations is given in Fig. 7, with the highest values of $4.65 \times 10^5 \text{ p cm}^{-3}$, 14.7 p cm^{-3} and $409 \mu\text{g m}^{-3}$ for submicrometer $\text{PN}_{0.005-3 \mu\text{m}}$, supermicrometer $\text{PN}_{0.5-20 \mu\text{m}}$ and PM_{10} , respectively.

Analysis of the time series of indoor and outdoor particle concentrations combined with nebulization therapy activities and drug using records, identified nebulization of three drugs (salbutamol, ipratropium and adrenaline) as a source of indoor particle production. A summary of the estimated PM_{10} , $\text{PN}_{0.005-3 \mu\text{m}}$ and $\text{PN}_{0.5-20 \mu\text{m}}$ emission rates during nebulization of these drugs, and the median peak concentrations, are presented in Table 2, from which it can be seen that the highest emission rates are from nebulized salbutamol (PM_{10} : 7.41 mg min^{-1} ; $\text{PN}_{0.005-3 \mu\text{m}}$: $3.96 \text{ p min}^{-1} \times 10^{11}$; $\text{PN}_{0.5-20 \mu\text{m}}$: $6.39 \text{ p min}^{-1} \times 10^8$).

3.5. Ventilation

The average and range of CO_2 concentration, temperature and relative humidity in the PICU were 433 (404–837) ppm, $22.8 (21.2-24.3) ^\circ\text{C}$ and $47.3 (33.3-59.4) \%$, respectively.

Based on particle concentrations in outdoor air and inside the ventilation duct, the average filtration efficiency of the primary filter of the air conditioning unit was estimated to be 57.5% and 40.0% for submicrometer PN and PM_{10} , respectively. Based on particle concentrations in the ventilation duct and the supply air, the average filtration efficiency of the HEPA filter was estimated to be 99.98% for submicrometer PN. The overall filtration efficiency of the two-stage filters was 99.99%, which is close to the design value of 100%.

Fig. 5. Example of time series of outdoor and supply air PN concentrations.

Fig. 6. Time series of indoor and outdoor PN_{0.005–3 µm} and indoor PN_{0.5–20 µm} concentrations (A, 5 August 2014), as well as indoor and outdoor PN_{0.005–3 µm} and PM₁₀ concentrations (B, 9 August 2014) in the PICU.

Fig. 7. Daily maximum indoor PN_{0.005–3 µm}, PN_{0.5–20 µm} and PM₁₀ concentrations in the PICU.

Table 2Peak concentration values (PV) and the estimated emission rates (ER) of PM₁₀ and PN_{0.005–3} µm and PN_{0.5–20} µm during nebulization therapy activities.

Drug	N	PM ₁₀		PN _{0.005–3}		PN _{0.5–20}	
		PV	ER	PV	ER	PV	ER
		(µg m ⁻³)	(mg min ⁻¹)	(p cm ⁻³ × 10 ³)	(p min ⁻¹ × 10 ¹¹)	(p cm ⁻³ × 10 ³)	(p min ⁻¹ × 10 ⁸)
Salbutamol	5	76*	7.41*	4.27*	3.96*	12.8*	6.39*
Ipratropium	1	40	5.43	2.47	2.39	N/A	N/A
Adrenaline	1	12	1.29	1.10	1.20	3	1.86

Note: N: sample number; *: median value; N/A: no data available.

The average velocity of the supply air varied between the outlets, and ranged from 0.5 m s⁻¹ to 1.4 m s⁻¹, with an overall average of 1.0 ± 0.3 m s⁻¹. The overall equivalent supply air flow rate was 5256 m³ per hour and the estimated total mechanical air exchange rate was 10.8 h⁻¹. These results provide evidence that the use of a two-stage filter system in this hospital was very effective in supplying clean air into the PICU. The main return air outlet was located in the middle between Bed 5 and Bed 6, from which about 32% of all return air was returned.

3.6. Particle transport

After noting PN concentration peaks associated with presumed secondary particle formation following floor cleaning with detergent, we recognized that we were able to use it as an opportunistic tracer of air movement within the PICU. Thus, the particle transport patterns in the PICU were assessed serendipitously by conducting simultaneous multi-location measurements of indoor submicrometer particle concentrations generated by the cleaning detergent.

The results showed that: 1) particles generated at all the beds, except Bed 1, could be transported across the whole center; 2) particles generated at any of the center locations could also be transported to all beds, except Bed 1 and 3) particles generated at any bed could be transported to other beds, except Bed 1 and 4) the concentration of transported particles was dependent on the distance between the two locations; however, particles generated at any bed, except Bed 1 and 2, were more easily transported to the center location. Examples of the particle concentration time series used to determine particle transport are given in Figs. 8, 9 and 10.

3.7. Indoor airborne bacteria and viruses

3.7.1. Indoor airborne bacteria

Eighty-two samples were processed for bacterial testing. Forty-nine samples were culture negative following processing of the primary sample and of these, only 18 remained sterile at the completion of the broth culture method. The average bacteria concentration was < 13 CFU m⁻³.

The most commonly isolated bacterial genus from both the primary and broth cultures were those referred to as skin commensals (Kong and Segre, 2012), including coagulase-negative *Staphylococcus* (primary culture 45.5%, broth culture 64.9%), *Micrococcus* species (primary culture 30.3%, broth culture 21.1%) and *Corynebacteria* species (primary culture 9.1%, broth culture 7.0%) (Table 3). Of the samples that were positive for bacterial growth in the primary culture, 69.7% grew the same organism in the broth culture, whereas only 40.4% of all culture positive broth cultures matched the growth from the primary cultures.

Presumptive bacterial pathogens were isolated from a total of seven samples, including four samples collected from beds and three from the common walkway corridor within the PICU ward. Bacterial counts of the pathogens isolated from the primary sample collection were low (≤ 5 CFU ml⁻¹). Following identification using the MALDI-TOF,

Fig. 8. Time series of indoor PN concentrations at two locations (Center and Bed 6), with particle source information (at Bed 5 or Bed 7, respectively).

pathogens were confirmed present in only three samples, with the remaining four being confirmed as skin commensals (n = 2), *Neisseria* species (n = 1) and a non-viable culture. *Pantoea dispersa* was isolated in the primary and broth cultures from Bed 3, and *Staphylococcus aureus* and *Enterobacter cloacae* were isolated from the primary cultures of the common walkway corridor and Bed 8, respectively (Table 4).

3.7.2. Indoor airborne viruses

Extraction of nucleic acids from all the air sample aliquots was conducted and these were tested for rhinoviruses, because of their expected ubiquity. No samples were positive for any rhinovirus.

3.8. Swab testing

Extraction of nucleic acids and testing for rhinoviruses, influenza

Fig. 9. Time series of indoor PN concentrations at three locations (Up: Center, Bed 2 and Bed 7) and four locations (Down: Center, Bed 2, Bed 5, Bed 7), with particle source information (at Bed 2 or Bed 7 or Bed 7 and Bed 8, respectively).

viruses, adenoviruses, RSV, HMPV and parechoviruses on all PICU-outbound nasal swabs was performed. Testing for HCoV and HPIVs was also performed for 60 nasal swabs. Viral RNA was detected in four swabs; swab#9 for HCoV-OC43, #11 and #12 for HMPV and #109 for HCoV-NL63.

Higher levels of detection had been hypothesised, since even healthy people will occasionally have a virus detectable. We also tested the extracts for the presence of a human gene to ensure that: 1) we were getting cells in the swabs as a sign of a satisfactory swabbing of the nares; and 2) that our assay reagents were performing as expected. All swabs tested positive for human mRNA using the UBE2D2 assay. Additionally, our positive and negative viral controls in each run functioned within specifications.

There were fewer swabs positive for a virus than we had expected, but all contained human cells as indicated by positive UBE2D2 RT-PCR results. We did not have the funds available to use virus concentrating columns. Their use may have yielded additional positives in air samples; however, the paucity of viruses among staff and visitors of patients suggests it would be unlikely.

4. Discussion and conclusions

Over a two-week period we measured simultaneously and continuously the indoor and outdoor particle mass and number concentration of the PICU, Royal Children's Hospital, Brisbane, Australia. We also collected indoor bioaerosol samples to determine the presence of pathogens. The indoor concentration levels, both particle number

Fig. 10. Time series of indoor PN concentrations at four locations (Up: Center, Bed 2, Bed 5 and Bed 8) and four locations (Down: Center, Bed 1, Bed 5, Bed 8), with particle source information (at between Center and Bed 8 or at Bed 5 or at Bed 1 or between Bed 5 and Bed 6, respectively).

Table 3

Bacterial pathogens isolated from aerosol sampling of the PICU, Royal Children's Hospital.

Collection date	Sample collection location	Primary culture results	CFU ml ⁻¹	Broth culture results
Day 2	C(4)	<i>Staphylococcus aureus</i>	100	NG
Day 4	B8	<i>Enterobacter cloacae</i>	200	Non-lactose fermenting gram negative bacillus ^a
Day 8	B3	<i>Pantoea dispersa</i>	500	<i>Pantoea dispersa</i>

Note: CFU ml⁻¹, colony forming unit per milliliter of sample; B, bed; C, central area of ward.

^a Isolate not stored, therefore identification could not be confirmed.

and mass, in this PICU were significantly lower than those in any other indoor environments in Brisbane, which we measured (e.g. residential houses, office buildings, schools, child care centers), except the bone bank building (He et al., 2014; Morawska et al., 2003; Quang et al., 2013; Morawska et al., 2009; Guo et al., 2010).

Partially due to the unique and sensitive nature of PICUs, only one study has reported both particle number and bioaerosol concentrations there. Li and Hou (2003) measured the time series of supply and room air total particles (particle size ranging from 0.1 to 5 μm) number concentrations during day time (08:00–18:00) for two days in a PICU in Taiwan. The study showed that the average supply air PN_{0.1–5 μm}

Table 4

Microbiological culture results of bioaerosol samples collected from the PICU, Royal Children's Hospital.

	Primary culture results (n = 82)	Broth culture results (n = 81) ^a
No growth	49	23
CNS	15	36
<i>Micrococcus</i> sp.	10	13
<i>Corynebacterium</i> sp.	3	4
<i>Streptococcus viridans</i>	2	1
<i>Bacillus</i> sp.	2	4
<i>Neisseria</i> sp.	1	1
Fungal sp.	2	0
Pathogen isolated ^b	3	1
Not viable	0	1

CNS, Coagulase negative Staphylococcus.

Five samples in both the primary culture and the broth culture had a polymicrobial infection with two species identified in each sample.

^a One collection had insufficient sample for processing.

^b Recognized pathogens include *Staphylococcus aureus* (n = 1), *Enterobacter cloacae* (n = 1), *Pantoea dispersa* (n = 2).

concentrations ranged from 0.04 to 1.85 p cm⁻³ (median of 0.45 p cm⁻³) and room air PN_{0.1–5 μm} concentrations, from 0.50 to 4.67 p cm⁻³ (median of 2.0 p cm⁻³). This implies that the average indoor PN_{0.1–5 μm} concentrations were about 3.4 times higher than those of supply air in that PICU. In our study the average indoor PN concentrations were about 17 times higher than those in supply air. However, it should be noted that it is difficult to compare the results from the two studies because of the different instruments used and the particle size range measured in the two studies.

Recently, Licina et al. (2016) estimated indoor PM₁₀ concentrations in a neonatal intensive care unit (NICU) based on year-long particle number concentration measurements (0.3–10 μm, 6 size groups) in a private hospital in the USA. They found that the time-averaged PM₁₀ concentrations in the individual baby rooms were generally low, not exceeding 5 μg m⁻³ (mean ± standard deviation = 2.9 ± 0.8 μg m⁻³), which they attributed to efficient particle filtration, effective building hygiene protocols and relatively low occupancy. Tang et al. (2009) and Gaidajis and Angelakoglou (2014) measured PM₁₀ concentrations in ICUs in Taiwan and Greece, respectively. The average PM₁₀ concentration values in ICUs were 14 μg m⁻³ in Taiwan and 59 μg m⁻³ in Greece. In contrast, this study showed that the average PM₁₀ value (1.0 ± 0.5 μg m⁻³) was significantly lower than the previous research in NICU or ICU environments.

Li and Hou (2003) measured bioaerosol concentrations in the PICU and reported that the average concentrations of fungi and bacteria ranged from 13 to 259 CFU m⁻³ (median: 41.5 CFU m⁻³) and from 35 to 156 CFU m⁻³ (median: 46.5 CFU m⁻³), respectively. However, there was no clear information regarding the identity of the microorganisms detected. Bacteria concentrations (< 13 CFU m⁻³) found in our study were lower than the values previously reported by Li and Hou (2003).

The results of this study show that nebulization therapy generated a large amount of submicrometer and supermicrometer particles, which were transported quickly within the PICU and led to substantial increases in the indoor PM₁₀ and PN levels. The moveable walls in the PICU may have partially aided in reducing particle transport between the beds, but not between the beds and the nurses station.

There may be three mechanisms of particle generation during nebulization therapy: from the drug, the drug solution, and the patient's breathing. For example, Roberts et al. (2006) reported that the use of nebulisers appears to be associated with the production of particles < 3 μm and, more importantly, that the bioaerosol concentration significantly increased from approximately 70 to 1300 CFU m⁻³ during nebulization therapy in an ICU in the UK. While nebulization of drugs is required to treat patients, due to the large production and efficient

movement of particles within the PICU, it is prudent to consider whether it could lead to unintended exposures to these particles among other patients and staff. Similarly, other patients and staff in the PICU may also be unnecessarily exposed to particles generated from the nebulized drug solution. Furthermore, particles generated from patients' breathing could also be considered a risk to other patients and staff in the PICU, as they may contain harmful bacterial and viral pathogens. However, the potential risk of nebulization may be reduced within this PICU as no rhinoviruses were detected in any of the samples tested during this study, despite being considered common viruses even among those without symptoms.

Based on the results, the following recommendations may be considered to mitigate aerosol generation and transmission: (1) for hospital designers: designing a better ventilation system or adding bed head removable return air inlets, which can be employed during nebulization therapy or when patients are coughing; (2) for managers and nurses: using screens or individual rooms to separate patients; (3) for cleaners: using low volatile organic compound emission detergents; (4) for nurses: making bed gently, and strictly using personal protective clothing (mask) during nebulization therapy or when patients are coughing.

To our knowledge, we have conducted the first comprehensive measurements of indoor/outdoor particle number, particle mass and indoor bioaerosol characteristics in a PICU environment. The average indoor PN and PM₁₀ concentrations in the PICU were substantially lower than outdoors. The contribution of outdoor particles to indoor particle concentrations was very low for both PN and PM₁₀. This result could be due to the two-stage filters (primary filter: fabric bag; secondary filter: HEPA filter) which achieve 99.99% filtration efficiency. However, regular maintenance of the ventilation system is needed to ensure the HEPA filters are in good working order.

There were strong indoor particle sources in the PICU, which could significantly increase indoor particle concentrations up to 100 times for PN and 68 times for PM₁₀. The main indoor sources for particle generation identified in the PICU were nebulization therapy, tracheal suction and cleaning activities. There were also some particle sources that were not identified, due to a lack of recorded activity information. Based on multipoint measurement data, the spatial variation of particle concentration in the PICU was low because of rapid transport of particles generated across the whole area of the PICU. Surprisingly, particles generated in the isolated single room (Bed 2) were also transported to other open areas or other beds. Despite the transport of particles from the isolation room to other areas of PICU, this study showed lower bioaerosol concentrations compared with the previous research in PICU environments. Therefore, the results of this study indicate that the likelihood of bioaerosols being transported from the isolation room to other areas of the PICU is small; however, it is still an infection control risk, especially in the absence of viruses being detected in airborne particles in our study.

The multipoint measurement database obtained from this study not only improved our understanding about the transport, mixing and dilution of the particles present in the ward, but also will be useful to validate indoor particle dynamic modelling. This might be further helpful to those formulating strategies for better ventilation of hospital wards, as well as infection or air indoor air pollutant control and management.

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Appendix A. Supplementary data

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