Airborne bacterial and PM characterization in intensive care units: correlations with physical control parameters

S. AlRayess1 · A. Sleiman2,3,4 · I. Alameddine1 · A. Abou Fayad2,3,4 · G. M. Matar2,3,4 · M. El-Fadel1,5

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Abstract
In this study, the spatial variation of airborne bacteria in intensive care units (ICUs) was characterized. Fine particulate matter and several physical parameters were also monitored including temperature and relative humidity. The results showed that the total bacterial load ranged between 20.4 and 134.3 CFU/m³ across the ICUs. Bacterial cultures of the collected samples did not isolate any multi-drug-resistant Gram-negative bacilli indicating the absence of such aerosolized pathogens in the ICUs. Meanwhile, particulate matter levels in several ICUs were found to exceed the international guidelines set for 24-h PM exposure. Moreover, examining bacterial load contribution by size suggested that bacteria with sizes less than 0.65 µm contributed the least to the total bacterial loads, while those with sizes between 0.65 and 1.1 µm contributed the most. A multiple linear regression model was also built to predict the bacterial loads in the ICUs. The regression analysis explained 77% of the variability observed in the measured bacterial concentrations. The model showed that the level of activity in the ICU rooms as well as its occupancy level had strong positive correlations with bacterial loads, while distance away from the patient had a non-linear relationship with measured loads. No statistically significant correlation was found between bacterial load and particulate matter concentrations.

Keywords Hospitals · Intensive care units · Indoor air quality · Airborne bacteria · Particulate matter

Introduction
Poor indoor air quality (IAQ) is associated with serious health implications. Some facilities, such as hospitals, are more critical than others in the presence of vulnerable patients. Physical and chemical characterizations of IAQ in hospitals have been widely reported (Lomboy et al. 2015; Loupa et al. 2016; Mohammadyan et al. 2018; Scheepers et al. 2017) with some efforts targeting bioaerosols (Asif et al. 2018; Baurès et al. 2018; Fu Shaw et al. 2018; Leung et al. 2016; Lindsley et al. 2010b; Blachere et al. 2009) that can cause respiratory infections requiring hospitalization (Kestler et al. 2018; Mullooly et al. 2007). The exposure of vulnerable patients to such pollutants can indeed negate the purpose of their hospital visit and is likely to extend hospital stays (Falsey et al. 2005; Lee et al. 2013; Volling et al. 2014). As such, several studies have probed into the health effects of compromised IAQ and highlighted both short-term (Tran et al. 2020) and long-term (Gola et al. 2019) hazards. The acute health effects range from mild irritations of the eyes and nose to severe syndromes such as headaches, throat conditions, asthma, and fatigue (Tran et al. 2020). Long-term exposure to poor air quality could also contribute to chronic respiratory and cardiac complications as well as increased risks of cancer (Gola et al. 2019). With the spread of the COVID-19 pandemic, the airborne transmission of bioaerosols has become more critical worldwide particularly that bioaerosols can remain in the air for a few hours (Morawska et al. 2020; Ningthoujam 2020) through droplets
that are expelled by coughing and sneezing or through suspended aerosols (<5 µm) (Kutter et al. 2018).

The ventilation system (natural, mechanical, or mixed) can play a key role in the transport of various pollutants in hospitals (Jung et al. 2015). Concurrently, temperature (T) and relative humidity (RH) are also known to affect the movement, decay, and settlement of various pollutants, especially bioaerosols (Murphy 2006). Variations in T and RH can activate or deactivate bioaerosols and exacerbate health risks (Vuurinen et al. 2020; Kameel and Khalil 2003; Murphy 2006). In turn, particulate matter (PM) contributes to the transport of bacterial and viral infections through coagulation (Annesi-Maesano et al., 2007) with several studies reporting high PM levels in hospitals (Ostro et al. 2009; Slezakova et al. 2012) due mostly to indoor-outdoor correlations, entraining outdoor pollutants to the indoors, and/or to inadequate air exchange, failing to filter indoor pollutants leading to their accumulation (Wang et al. 2006). Similarly, bacterial loads have been measured at different locations within hospitals (Cabo Verde et al. 2015; Asif et al. 2018), with one study only targeting patients’ rooms in intensive care units (ICUs) with measurements only done at a single location (O’Neil et al. 2017).

Empirical data ascertain that ventilation systems play a critical role in regulating IAQ. Božić and Ilić (2019) observed that natural ventilation and fan-coiled units aggravated the concentration of indoor PM and fungi, while air-handling units reduced their concentrations. These observations were consistent with earlier reported assertions that poorly maintained heating, ventilation, and air conditioning (HVAC) systems are primary catalysts for the multiplication of suspended microorganisms within offices (Burge et al. 2000; Wu et al. 2005; Maclntosh et al. 2006), implying that IAQ control strategies cannot be limited to the pollutant source removal alone but also to the modification and maintenance of HVAC systems (Božić and Ilić, 2019) or the deployment of active ionization technology (Sidhu 2018). Recent work has underscored the importance of characterizing the spatio-temporal variability in the concentrations of microorganisms in the indoor air across a hospital. The temporal variability in indoor air quality has been well studied. For example, Zaman et al. (2021) highlighted the temporal variabilities in indoor PM concentrations across a year, with the colder season associated with higher PM, microorganisms, and CO₂ levels as compared to the warmer season. Both Chamseddine et al. (2019) and Zaman et al. (2021) offered possible explanations for these seasonal variations; both cited that increased indoor activity during the cold season increased the rate of pollutants influx, ultimately causing the observed surges in concentrations. It should be noted that the impact of seasonality on indoor air quality is not limited to the hospital setting; several studies have reported similar observations for residential settings (Abdel-Salam 2021) and in learning institutions (Deng and Lau 2019; Stamp et al. 2022). In comparison, the spatial variability of microorganism levels in hospitals remains less studied. Yet, Lee et al. (2020) have reported that suspended PM and microorganism levels measured in more critical health units, such as ICUs, can often be higher than those measured in other open spaces like corridors and reception areas.

In this study, we present a first attempt at examining the spatial variation of airborne bacterial levels in ICU rooms and evaluate their variability as a function of particle size to provide an understanding of factors affecting bacterial loads in ICUs. Concurrently, we monitored particulate matter (PM₁₀, PM₂.₅) and measured several physical parameters (temperature, relative humidity, distance away from patient, and level of activity). We then developed multivariate regression models (MLRs) using correlations between pollutant levels and physical parameters.

Materials and methods

Study design and monitoring program

The monitoring program was implemented for 10 different ICU patients during night hours. All samples were collected between 19:00 and 04:00. Sampling was done in one patient room per night and the total duration of sample collection in each room was approximately 1 h. All monitored ICUs were located at the American University of Beirut Medical Center (AUBMC). Sampling was done following receiving the approval of the University Institutional Review Board (IRB). The sampling period spanned from June to September 2019. Patients admitted to the ICU, mostly suffering from bacterial infection or physical traumas, were randomly selected. Nearly 70% of the approached patients accepted to take part in this study. The monitored parameters included the total bacterial load (TBL) with corresponding distribution of bacteria by their diameter size, the Gram-negative bacterial load, particulate matter (PM₁₀ and PM₂.₅) concentrations, temperature (T), and relative humidity (RH). Additionally, the occupancy, the level of activity, and the room volume were recorded. Note that sampling was conducted in 4 ICU rooms. Three rooms were identical with an average volume of 40 m³ (L = 4.5 m, W = 3.5 m, H = 2.8 m), while the fourth was a suite room with a volume of 76 m³ (L = 7.8 m, W = 3.5 m, H = 2.8 m). The ventilation system in the hospital is fully mechanical with no windows in the ICU. The airflow within the ICU was reported by the mechanical department to range between 300 and 350 ft³/min, with an estimated average air change per hour (ACH) of 14. These are comparable to those reported in the literature (Saran et al. 2020).
A Six Stage Microbial Andersen Cascade Impactor (TISH Environmental Model TE-10–800) was used for the fractionation of bacteria to simulate various stages of the human respiratory track. The diameter cut-offs for the 6 stages were 0.65, 1.1, 2.1, 3.3, 4.7, and 7 µm. A volumetric sampling approach was followed to measure the concentrations of viable bacterial loads inside the ICU rooms. The impactor’s 12 Volts vacuum pumps were calibrated to 28.3 L/min (1ft³/min) at the beginning of every sampling round using a rotameter airflow meter with a capacity of 60 L/min (~2.1 ft³/min). In each room, six samples were collected at the breathing level of 1.5 m for a duration of 20 min, as recommended by Hiwar et al. (2021). The sampling was consistent with the World Health Organization (2020) guidelines on sampling and analysis methods for indoor pollutants. Two simultaneous, 20-min samples were collected at 0.5 and 1.5 m away from the patient and tested for total bacteria load (TBL). Subsequently, another two simultaneous, 20-min samples at 1 and 2 m away from the patient were recorded. Two simultaneous, 20-min samples at 0.5 and 1.5 m were also taken and tested for Gram-negative Bacteria. Two additional samples at distance of 0.5 and 1.5 m were also collected in the last two ICU rooms to measure the concentration of total bacteria resistant to meropenem. Note that all distances were measured from the patient’s face to the center of the Andersen impactor horizontally, while the concentrations of TBL were estimated as the sum of colony forming units found across the six stages of the impactor. The percent bacterial load contribution (BLC) for each size was calculated by dividing each particle size concentration with its corresponding TBL. This number represents the percentage contribution of each size to the total concentration and allows for a standard comparison of sizes across different samples. The effectiveness of the sampling protocol has been reported in previous studies (Erdogan et al. 2009; Kim et al. 2010).

For each room, 24 glass petri dishes were prepared by pipetting 27 mL of Tryptic Soy Agar and autoclaving them, while an additional 12 petri dishes were filled with MacConkey agar for testing for Gram-negative bacteria. Following sample collection, the plates were incubated at 37 °C for 18–24 h after which the colonies formed were counted and reported. The final concentrations were adjusted based on the volume extracted during the sampling period of 20 min as expressed in Eq. 1.

\[
TBL = \frac{C \times 1000}{V}
\]

where \( V = 566 \text{ L} \) is the volume of air sampled in 20 min, \( C \) is the bacterial count, and \( TBL \) is the total bacterial load expressed in number of colony forming units (CFU) per 1 m³ of air.

During the sampling process in an ICU room in the hospital (Fig. 1), the door was always kept closed. Also, the pumps were activated from outside the rooms 10 min after they were installed in their sampling locations to minimize the effect of any disturbance that might be created during their installation. In rooms where nurses had to enter, the number of trips and occupancy inside the room was recorded (i.e., presence of a private nurse). Most ICU rooms were occupied by only one patient, except for two rooms (Room ID 3 and 6 in Fig. 2), where a private nurse was always present. The nurse was asked to remain seated during the sampling period. As for the regular hospital nurses’ trips, most were short (<1 min).

The \( \text{PM}_{2.5} \) and \( \text{PM}_{10} \) levels were monitored using a factory-calibrated portable TSI DustTrak™ II Aerosol Monitor (Model 8532, TSI Corporation, Shoreview, USA) with a log interval of 1 min. The T and RH inside the patients’ rooms were recorded over a period of 20 min using a portable Langan analyzer (model L76n) with a log interval of 10 s. During the monitoring period, occupancy levels and the number of coughs were recorded to account for their potential effect on bacteria shedding, \( \text{PM}_{2.5} \) and \( \text{PM}_{10} \) concentrations, and airflow.
Statistical analysis

Correlations, ANOVA, and multiple regression were used to assess the importance of several factors in predicting bacterial concentrations in the ICU rooms. The average indoor PM$_{2.5}$ and PM$_{10}$ levels were compared with IAQ guidelines (WHO 2021). Pearson’s correlation coefficient was used to quantify the correlations between the indoor air quality variables (PM, TBL) and several predictors including T, RH, occupancy, and the number of nurses’ trips. A stepwise multiple linear regression model was also developed to predict TBL from the predictors measured in each room. The statistical analysis was performed using the R software (R Core Team 2022).

Isolates collection and broth micro-dilution

Luria agar plates supplemented with 1 µg/mL of meropenem were prepared for the detection of the presence of meropenem-resistant microorganisms in the ICU rooms. The Viable 6-stage Andersen impactor was used to test the presence of airborne meropenem-resistant microorganisms. Minimum inhibitory concentrations were determined using the broth micro-dilution against ertapenem, meropenem, imipenem, gentamicin, ciprofloxacin, cefepime, vancomycin, and dalfo-pristin quinupristin (CLSI). Serial dilution of each antibiotic was prepared in Cation-adjusted Mueller Hinton broth in 96-well plates between columns 1 and 10. Column 1 had a concentration of 128 µg/mL while column 10 had a concentration of 0.25 µg/mL. Column 11 served as a positive control, while column 12 served as a negative control. Bacteria were adjusted in Cation-adjusted Mueller Hinton broth to a turbidity equal to that of the 0.5 McFarland standard, followed by a dilution to reach 5 × 10$^6$ CFU/mL. From the latter, 10 µL was added to each well between columns 1 and 11, leading to a final bacterial concentration of 5 × 10$^5$ CFU/mL. Each plate was then incubated at 37 ºC for 18–24 h, and the results were determined based on turbidity.

Bacterial cultures were grown overnight on Columbia sheep blood agar (Becton Dickinson, Heidelberg, Germany) at 37 ºC and subjected to ethanol-formic acid extraction according to the following protocol. One full 1 µL sterile loop of bacterial sample was suspended in 300 µL of sterile water and mixed with 900 µL of absolute ethanol. Samples were centrifuged at 12,000 g for 2 min and the supernatant was discarded. The pellet was re-suspended in 50 µL of 70% formic acid and 50 µL of acetonitrile (Sigma) and centrifuged at 12,000 g for 2 min. The supernatant was collected and stored at 20 ºC. A 1 µL of each bacterial extract was spotted onto a MALDI target plate (MSP 96 target ground steel; Bruker Daltonics, Bremen, Germany) and air-dried at room temperature. Each spotted sample was then overlaid with 1 µL of a saturated matrix solution (α-cyano-4-hydroxy-cinnamic acid; Bruker Daltonics) in 50% acetonitrile and 2.5% trifluoroacetic acid then air-dried. Samples were measured on Microflex-LT system (Bruker Daltonik, Bremen, Germany).

Results and discussion

Monitoring program

The measured T in the ICU rooms ranged from 22.0 to 23.5 ºC (mean = 22.5 ºC, SD = 0.46 ºC), while RH ranged from 57.2 to 63.5% (mean = 61.5%, SD = 1.94%). The low variability in T and RH is expected in ICU rooms due to the controlled mechanical ventilation and absence of natural ventilation.

PM$_{10}$ levels ranged from 10 to 65 µg/m$^3$ (mean = 33 µg/m$^3$, SD = 17.8 µg/m$^3$), while PM$_{2.5}$ levels ranged from 10 to 54 µg/m$^3$ (mean = 30 µg/m$^3$, SD = 16.8 µg/m$^3$). The measured values in several ICU rooms exceeded international...
The levels of PM$_{10}$ and PM$_{2.5}$ were found to be highly correlated ($r = 0.98$). Moreover, most of the measured PM$_{10}$ was in the form of PM$_{2.5}$ (mean of PM$_{2.5}$/PM$_{10}$ ratio = 0.90, SD = 0.1). Fine particles (PM$_{2.5}$) tend to remain permanently suspended and slightly affected by resuspension (Hospodsky et al., 2012) which explains the low correlation (Table 1) observed between PM concentrations and the level of activity in each room (number of trips and occupancy rate). Note that the measured PM values fell within the range reported in the literature for measurements collected at different locations within hospitals (Table 2).

The TBL concentrations (see Supplemental Material) ranged from 20.4 to 134.3 CFU/m$^3$ (mean = 66.43 CFU/m$^3$, SD = 35.20 CFU/m$^3$). These values are at the lower end of reported literature measurement at different locations within hospitals (Table 3). Osman et al. (2018) conducted bimonthly (twice per month) sampling for a year in Egypt across different hospital units, including ICU wards. Their study reported TBL ranging from 118 to 1124 CFU/m$^3$ across different hospital units, including ICU wards. These values are at the lower end of reported literature measurement at different locations within hospitals (Table 3). Osman et al. (2018) conducted bimonthly (twice per month) sampling for a year in Egypt across different hospital units, including ICU wards. Their study reported TBL ranging from 118 to 1124 CFU/m$^3$ (mean = 512 CFU/m$^3$, SD = 425 CFU/m$^3$), which is within the same order of magnitude, but higher than the TBL reported in this study. One possible reason for the difference is that Osman et al. (2018) sampled the ICU wards, which are more dynamic and less frequently cleaned as compared to ICU patient rooms. Other reasons could be (1) the difference in the sampling timing (day hours vs night hours), as day hours are usually more crowded with staff and visitors; and (2) the shorter sampling duration they adopted (5 min vs 20 min) that could lead to higher margins of error.

Correlations between TBL and the measured T ($r = -0.31$, significant at the 10% level) and RH ($r = -0.15$) (Table 4) were not statistically significant, as both physical parameters remained relatively constant in patient rooms as a result of mechanical ventilation. Note that Osman et al. (2018) reported negative correlations between TBL and physical parameters; yet these correlations could have been due to their sampling in less controlled areas within a hospital environment (i.e., ICU ward and admission department). As for the level of activity, the number of nurses’ trips was found to be highly correlated with the measured concentrations of airborne bacteria ($r = 0.86$; $p$-value $< 0.05$) (Fig. 3). Also, significant differences in the mean TBL were observed as a function of the number of trips (ANOVA, $F$-value = 99.47; $p$-value $< 0.05$). The results from the multi-comparison $t$-tests, with Holm’s correction, showed that the mean concentration when no trips occurred was statistically lower than all other levels, where at least one trip was conducted (mean TBL for no trips was 39.87 CFU/m$^3$, $p$-value $< 0.05$). Meanwhile, the mean concentration when three or more trips occurred was significantly higher than the rest (mean = 117.3 CFU/m$^3$, $p$-value $< 0.05$). Similarly, the occupancy level was found to affect the measured bacterial concentrations in the air, since additional occupants can be bacteria sources and their activity may lead to the resuspension of settled bacteria. Hathway et al. (2011) conducted a 5-day air sampling campaign in a respiratory ward and reported that the ward activity level was highly correlated with the airborne concentrations of bacteria, while the presence of sedentary visitors was not. Pankhurst et al. (2012) reported that the number of people present in an operating

### Table 1 Correlation of PM concentrations with the level of activity

| Indicator | PM$_{10}$ | PM$_{2.5}$ |
|-----------|-----------|------------|
| Number of trips | $r^a=0.30$ | $r^a=0.26$ |
| Occupancy | $r^b=0.13$ | $r^b=0.11$ |

$^a$Pearson’s correlation coefficient  
$^b$Significant to the 10% level

### Table 2 PM concentrations in hospitals

| Reference | PM$_{2.5}$ (µg/m$^3$) | PM$_{10}$ (µg/m$^3$) |
|-----------|-----------------------|---------------------|
|           | Mean | SD | Range | Mean | SD | Range |
| **This study** | | | | | | |
| Asghar et al. (2022) | 30  | 16.8 | 10–54 | 33  | 17.8 | 10–65 |
| Danesh Yazdi et al. (2021) | 10.2 | - | 31 | - | - | - |
| Baurès et al. (2018) | 1.6  | - | 0–45.4 | 12  | - | - |
| Scheepers et al. (2017) | 9.8  | - | - | - | - | - |
| Powell et al. (2015) | 51.5 | 21.6 | 15–122 | 91.8 | 61.3 | 28–186 |
| Jung et al. (2015) | 14.4 | 15.9 | - | 25.2 | 17.2 | - |
| Slezakova et al. (2012) | 23.4 | - | 10.5–41.9 | 30.8 | - | 13–58.8 |
| Wan et al. (2011) | 1.0  | - | 0.1–8.4 | 10  | - | 0.8–55.6 |
| Ostro et al. (2009) | 19  | 15 | 0–100 | - | - | - |
| Wang et al. (2006) | 128.1 | - | 61.7–250 | 99  | - | 40.9–214.9 |
| Nardini et al. (2004) | 1.6  | 0.9 | 0–110 | - | - | - |
theater significantly increases TBL. However, their study did not differentiate between occupancy and level of activity. In our study, the mean TBL in rooms with one versus two occupants was found to be statistically different ($p$-value < 0.05), with the mean level in the former measured at 31.7 CFU/m$^3$, while the latter had a mean concentration of 59.8 CFU/m$^3$. On the other hand, the correlation between TBL and the distance away from the patient was found to be negative as expected; however, it had a weak correlation ($r = -0.12$; $p$-value = 0.47). TBL levels were found to show a constant drop up to 1.5 m away from the patient. Yet, TBL was found to increase again at 2 m (Fig. 3). This could be due to the proximity of the impactor to the door at 2 m, which could have resulted in the samples being affected by infiltration from the ICU common ward. Osman et al. (2018) reported high TBL in ICU wards (mean of 512 CFU/m$^3$), while the literature consistently reported higher TBL in different hospitals wards and common area (Table 3) (Asif et al. 2018; Mirhoseini et al. 2015; Sudharsanam et al. 2012). In addition, Pankhurst et al. (2012) reported that TBL can increase by 50% when comparing a closed operating theater to an “open door” operating theater, supporting the fact that nearby wards can be a major source of airborne bacteria. It is also important to note that TBL and PM levels were not strongly correlated in this study, which may suggest that the two have different sources.

**Bacterial load contribution by size**

Examining the bacteria load contributions (BLC) for each size (see Supplemental Material), a significant difference in their mean contribution by size is evident (ANOVA $F$-value = 13.41; $p$-value < 0.05). The results from the multi-comparison $t$-tests with Holm’s correction showed that the mean BLC for the bacterial sizes less than 0.65 micron.
(mean BLC = 5%) was statistically lower than all other bacterial sizes \((p\text{-value} < 0.05)\). The mean BLC for bacteria with sizes between 0.65 and 1.1 microns (mean BLC = 33%) was significantly higher than the rest of the bacterial size groups \((p\text{-value} < 0.05)\) (Figs. 4 and 5). Consistent with literature-reported data (Clauß, 2015), the contributions of all other sizes did not exhibit a statistically significant difference in their mean contribution.

A correlation analysis was conducted between measured bacterial loads by size and the different physical, PM, and occupancy variables measured in each ICU. The bacteria were divided into 3 categories, namely the small size category with diameters < 2.1 µm, the medium size category with diameters between 2.1 and 4.7 µm, and the large category for those with diameters > 4.7 µm. There was no correlation between the concentrations of small bacterial particles and distance away from the patient \((r = 0)\), suggesting that small diameter bacterial concentration is constant across the room (well-mixed). A weak negative correlation was found with the bacteria in the

![Fig. 3](image-url)  
**Fig. 3** TBL as a function of a number of trips, b occupancy, and c distance away from patient

![Fig. 4](image-url)  
**Fig. 4** Bacteria concentration by size and room

| Room ID | 0.65 µm | 1.1 µm | 2.1 µm | 3.3 µm | 4.7 µm | 7 µm |
|---------|---------|-------|-------|-------|-------|------|
| 1       | 51.8    |       |       |       |       |      |
| 2       |         | 193.8 |       |       |       |      |
| 3       |         |       | 231.2 |       |       |      |
| 4       | 448.8   |       |       | 360.4 |       |      |
| 5       |         |       |       | 258.4 |       |      |
| 6       |         |       |       |       | 304.3 |      |
| 7       |         |       |       |       |       | 102  |
| 8       |         |       |       |       |       | 149.6|
| 9       |         |       |       |       |       |      |
| 10      |         |       |       |       |       |      |
larger bacterial size group (Table 5). These results indicate that the heavier the bacteria, the higher the probability that it will settle with distance. This is consistent with the fact that gravitational velocity is proportional to the squared particle diameter (Seinfeld and Pandis 2006). As for the correlation between the bacterial concentrations and the number of trips by the nursing staff, the lowest correlation was found for the small-sized bacteria, which also supports the idea that these light particles tend to be well mixed and are the least affected by resuspension. Strong positive correlations were found between the number of trips on one hand and the medium- and large-sized particles concentrations on the other. This highlights the potentially important role that resuspension (due to increased activity) may have on these two sizes. The occupancy rate had a positive correlation with bacterial concentration irrespective of size. The correlation between bacterial concentrations on one hand and T and RH on the other showed that these were not significant because of the small fluctuations of the latter in the ICU rooms. The correlations between the concentrations of the different bacterial sizes and the measured PM concentrations were low for the same reasons discussed previously. Table 6 summarizes the correlations between the bacterial concentrations by size and the measured physical parameters.

### TBL regression analysis

A regression model was developed to predict the measured TBL levels as a function of the room characteristics and occupancy levels (Table 6). The model showed that distance away from the patient and its squared value (to account for the increase in concentrations at 2 m) along with its occupancy level and the number of trips to the room were strong predictors of TBL. Consistent with the reported literature, the number of trips was the most significant factor due to the potential increase in resuspension and its impact on the airborne bacterial concentration (Chen 2009; Hospodsky et al. 2012). In addition, O’Neil et al. (2017) analyzed different activities within ICUs and identified those that can generate significant amounts of airborne bacteria. Nurses could be a bacteria source and thus their presence may increase TBL. Additional work on the DNA of the collected bacteria is needed to determine their actual sources. Occupancy was

### Table 5 Correlations of bacterial concentrations with physical parameters

| Indicator            | Concentration of small particles (< 2.1 µm) | Concentration of medium particles (2.1 ≤ d ≤ 4.7 µm) | Concentration of large particles (> 4.7 µm) |
|----------------------|-------------------------------------------|----------------------------------------------------|------------------------------------------|
| Temperature          | −0.24 b                                   | −0.13                                              | −0.11 a                                  |
| Relative humidity    | −0.05                                     | −0.02                                              | −0.20                                    |
| Distance             | 0.001                                     | −0.08                                              | −0.15                                    |
| Number of trips      | 0.34 c                                    | 0.57 c                                             | 0.50 c                                   |
| Occupancy            | 0.18                                      | 0.38 c                                             | 0.49 c                                   |
| PM_{10}              | 0.14                                      | 0.18                                               | −0.30 c                                  |
| PM_{2.5}             | 0.16                                      | 0.27 b                                             | −0.28 b                                  |

*a* Significant to the 10% level  
*b* Significant to the 5% level  
*c* Significant to the 1% level

### Table 6 Regression model parameters for total bacterial load (TBL)

| Variable       | Unit    | Estimate | t-statistic | p-value |
|----------------|---------|----------|-------------|---------|
| Intercept      | CFU/m³  | 57.12 a  | 3.298       | 0.00234 |
| Distance (D)   | m       | −66.003 b| −2.41       | 0.0217  |
| Distance squared (D²) | m²     | 25.785 b | 2.372       | 0.02369 |
| Number of trips (Tₚ) |        | 21.487 c | 8.05        | 2.74E−09 |
| Occupancy (O)  |         | 15.597 b | 2.111       | 0.04245 |

\[ R^2 = 0.774 \]

\[ \text{TBL} = 57.120 - 66.003 \, \text{D} + 25.875 \, \text{D}^2 + 21.487 \, \text{Tₚ} + 15.597 \, \text{O} \]

*a* Significant to the 10% level  
*b* Significant to the 5% level  
*c* Significant to the 1% level

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![Distribution of bacteria by diameter size](image-url)
also found to be a significant predictor of TBL which is expected given that an additional occupant could emit bacteria through breathing, coughing, sneezing, or talking. As for distance away from the patient, the relationship is expected to be negative as the concentration should decrease when moving further away from the patient. Yet in our results, we had to account for a non-linear relationship with distance (distance squared term) to account for the observed increase in concentrations at 2 m that is probably attributed to bacteria entering from the ICU common ward. Overall, the performance of the model was good with an adjusted $R^2$ of 0.77 and showed no bias (0%) (Table 5). Note that the model did not account for the fact that each patient had a different shedding rate. Measuring the shedding rate of each can be done by taking surface samples from the patient’s mouth or having the patient exhale on an agar plate which were outside the scope of this study. Another source of uncertainty was the lack of continuous data on the airflow in each room, as the management was only able to provide a general range for the airflow (300–350 ft$^3$/min) within the hospital, which can significantly affect the spatial distribution of PM and airborne bacteria by creating regions of accumulation and dilution.

**Microbiological characteristics for resistant bacteria**

The samples collected on MacConkey agars did not yield bacterial growth; hence, Gram-negative bacteria were absent from the indoor environment in the ICU. As for the sampling of resistant bacteria in the last two rooms, twelve isolates were obtained from the Luria agar plates supplemented with 1 µg/mL of meropenem. Using MALDI-TOF mass spectrometry, four isolates were identified as *Staphylococcus hominis*, 4 as *Staphylococcus haemolyticus*, 2 as *Staphylococcus epidermidis*, 1 as *Corynebacterium afermentans*, and 1 as *Brevundimonas diminuta* (Table 7).

Broth micro-dilution results showed that 50% of the isolates were susceptible to gentamicin, 60% were susceptible to ciprofloxacin, and 100% were susceptible to vancomycin and dalfopristin quinupristin. However, for meropenem, ertapenem, imipenem, and ceftazidime, their breakpoints were not specified according to the CLSI and EUCAST guidelines (Table 8). Coagulase-negative staphylococci (CoNS), such as *Staphylococcus epidermidis*, *Staphylococcus hominis*, and *Staphylococcus haemolyticus*, are considered part of the skin normal flora (Garza-González et al. 2011). However, these species are among the most causative agents of hospital-acquired infections in ICUs (Fitzpatrick et al. 2002). Among the recovered isolates, gentamicin and ciprofloxacin resistance was detected. Such resistance imposes a serious

| Table 7 Identification of isolates |
|-----------------------------------|
| Isolate code | Species | Isolate code | Species |
| 1            | *Corynebacterium afermentans* | 7            | *Staphylococcus haemolyticus* |
| 2            | *Staphylococcus hominis*     | 8            | *Staphylococcus haemolyticus* |
| 3            | *Staphylococcus epidermidis* | 9            | *Staphylococcus haemolyticus* |
| 4            | *Staphylococcus epidermidis* | 10           | *Staphylococcus hominis*     |
| 5            | *Staphylococcus hominis*     | 11           | *Staphylococcus haemolyticus* |
| 6            | *Staphylococcus hominis*     | 12           | *Brevundimonas diminuta*     |

| Table 8 Isolates MIC against various antibiotics |
|-----------------------------------------------|
| MIC (µg/mL)                                   |
| Isolates | Mer | Ert | Imi | Cef | Gen | Cip | Van | DQ   |
|----------|-----|-----|-----|-----|-----|-----|-----|------|
| 2        | 0.5 | 4   | <0.125 | 4 | <0.125 | S | <0.125 | S | <0.125 | S |
| 3        | 1   | 16  | 0.5  | 8  | <0.125 | S | 0.25  | S | 4    | S |
| 4        | >128 | 8  | 0.5  | 4  | >128  | R | 0.5   | S | 4    | S |
| 5        | 4   | 128 | 16   | 128 | 0.25 | S | <0.125 | S | 2    | S |
| 6        | 8   | >128 | 16  | 128 | <0.125 | S | 0.25  | S | 2    | S |
| 7        | 32  | >128 | 1   | >128 | 128  | R | 8     | R | 2    | S |
| 8        | 4   | 16  | <0.125 | 16 | 8    | I | 1     | S | 0.5  | S |
| 9        | 16  | >128 | 32  | >128 | 128  | R | 8     | R | 2    | S |
| 10       | 4   | 32  | <0.125 | 4 | <0.125 | S | 4     | R | 2    | S |
| 11       | 32  | >128 | 128  | >128 | 128  | R | 128   | R | 2    | S |
threat, if one of these isolates were acquired by a patient from a healthcare worker. Therefore, healthcare professionals must take good care of their skin hygiene, to halt a possible transmission of resistant skin flora microorganism to their patients.

MIC, minimum inhibitory concentration; Mer, meropenem; Ert, ertapenem; Imi, imipenem; Cef, cefepime; Gen, gentamicin; Cip, ciprofloxacin; Van, vancomycin; DQ, dalfopristin quinupristin; S, susceptible I, intermediate R, resistance.

While we did not isolate multi-drug-resistant Gram-negative bacilli indicating the absence of such aerosolized pathogens in the ICUs, the results still raise concerns (mostly around nosocomial infections) requiring mitigation measures that can reduce concentrations of airborne contaminants.

Conclusion

We present a first attempt at characterizing the spatial variation of total bacterial load in ICU rooms while concurrently monitoring particulate matter (PM$_{2.5}$, PM$_{10}$), temperature (T), and relative humidity (RH) at several distances from patients. In parallel, we recorded the room occupancy and the number of nurses’ trips inside the room. The latter was found to be an important potential contributor affecting airborne bacterial concentrations along with the distance from the source. Several antibiotic-resistant bacterial species were identified, and a regression model was developed to predict concentrations at several points in a typical ICU room. Several mitigation measures can be implemented to better control biological air quality within ICUs including regular surface cleaning to remove settled PM and bioaerosols, along with reducing air disturbance within the room through limited occupancy and in–out trips. Medical protocols should encourage nurses to perform as many activities as they can through one room entry, which can significantly reduce the resuspension of bacteria. In addition, hospitals must adhere to well-established best practices and standards through regular maintenance of ducts, replacement of filters, monitoring of air quality, and UV disinfection.

Managing IAQ is an integrated approach that encompasses various stakeholders towards developing an environmental management plan with adequate resources for monitoring and feedback and to raise awareness of hospital’s staff regarding the importance of IAQ in protecting patients and occupants’ health. A common challenge to a proper implementation of such a plan in hospitals is the lack of standards for IAQ although benchmark guidelines have been reported (Capolongo et al. 2017).

Additional work to build on our findings can target CFD model simulations along with DNA analysis to identify sources of resistant bacteria and enhance the understanding of bioaerosols movement within hospitals in general and ICUs in particular.

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Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

Ethics approval This study involves human subjects and was approved by the Institutional Review Board (IRB) at the American University of Beirut Medical Center. All patients provided written informed consents prior to indoor air sample collection. The field sampling did not replace or obstruct routine medical care procedures and institutional protocols at the hospital.

Conflict of interest The authors declare that they have no known conflict of interests that could influence the work reported in this paper.

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