AMERICAN UNIVERSITY OF BEIRUT

INDOOR AIR QUALITY IN HOSPITALS: PM AND AIRBORNE BACTERIA

by
SAMER ABDO ALRAYESS

A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Engineering
to the Department of Civil and Environmental Engineering
of the Faculty of Engineering and Architecture
at the American University of Beirut

Beirut, Lebanon
June 2020

AMERICAN UNIVERSITY OF BEIRUT

INDOOR AIR QUALITY IN HOSPITALS: PM AND AIRBORNE BACTERIA

by

SAMER ABDO ALRAYESS

Approved by:

MUTASEM EL-FADEL

Dr. Mutasem El- Fadel, Professor — Advisor
Department of Civil and Environmental Engineering, AUB

GHASSAN MATAR

Dr. Ghassan Matar, Professor — Member of Committee
Department of Experimental Pathology,
Immunology and Microbiology, AUB

ANTOINE ABOU FAYYAD

Dr. Antoine Abou Fayyad, Assistant Professor — Member of Committee
Department of Experimental Pathology,
Immunology and Microbiology, AUB

IBRAHIM ALAMEDDINE

Dr. Ibrahim Alameddine, Assistant Professor — Member of Committee
Department of Civil and Environmental Engineering, AUB

Date of thesis defense: June 12, 2020

AMERICAN UNIVERSITY OF BEIRUT

THESIS, DISSERTATION, PROJECT RELEASE FORM

Student Name: AlRayess Samer Abdo

X Master's Thesis Master's Project Doctoral Dissertation

I authorize the American University of Beirut to: (a) reproduce hard or electronic copies of my thesis, dissertation, or project; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes.

I authorize the American University of Beirut, to: (a) reproduce hard or electronic copies of it; (b) include such copies in the archives and digital repositories of the University; and (c) make freely available such copies to third parties for research or educational purposes
after:

One ---- year from the date of submission of my thesis, dissertation, or project.
Two ---- years from the date of submission of my thesis, dissertation, or project.
Three ---- ✓ years from the date of submission of my thesis, dissertation, or project.

_____

Signature Date June 29th, 2020

Samer AlRayess

ACKNOWLEDGMENTS

I would like to thank my advisor Prof. Mutassem El-Fadel for his great guidance, help, encouragement and advice through the preparation phase, sampling and writing process of this thesis.

I am also thankful for my thesis committee members Dr. Ghassan Matar, Dr. Ibrahim Alameddine and Dr. Antoine Abou Fayyad for their help and feedback throughout all this work.

I remain grateful to Mr. Ahmad Sleiman and Mr. Bassam Hafi from the Experimental Pathology, Immunology, & Microbiology department at AUB for their support in the laboratory analysis and constructive input.

I would like to thank Dr. Ashraf Chamseddine for his help and assistance especially in kicking off my thesis samples'collection.

Also, I want to thank my family and friends for their continuous moral support through the duration of my study.

Lastly, special thanks are extended to Dar Al-Handasah (Shair & Partners) for its support to the graduate programs in Engineering at the American University of Beirut.

AN ABSTRACT OF THE THESIS OF

Samer Abdo AlRayess for Master of Engineering
Major: Environmental and Water Resources Engineering

Title: Indoor air quality in hospitals: PM and airborne bacteria

The effect of air quality on personal exposure and human health is often more pronounced indoors than outdoors because people spend most of their time inside. In this context, hospitals represent a sensitive environment with highly vulnerable individuals. In this study, indoor air quality (IAQ) is characterized in Intensive Care Units (ICUs) with emphasis on assessing the levels of particulate matter (PM_{10}, $PM_{2.5}$) and airborne bacterial levels with corresponding diameter sizes, while also characterizing physical parameters including temperature, relative humidity, distance away from patient and level of activity. Correlations between measured pollutant levels and physical parameters were quantified and used to develop representative multivariate regression models (MLRs) that predict the pollution levels. Measured concentrations of PM_{10}, $PM_{2.5}$ and total bacteria ranged from 10 to 65 µg/m^3, 10 to 54 µg/m^3 and 20.4 to 134.3 CFU/m^3, respectively. These levels exceeded in many instances international guidelines set for IAQ. Total Bacterial Loads (TBL) varied significantly as a function of room occupancy and the number of trips conducted by the nursing crew. While TBL and PM levels exhibited a weak correlation indicating potential different sources, the concentrations of the heavy bacteria showed a positive correlation with the level of activity in the room. The TBL regression model was able to explain 77% of the variability observed in the measured bacterial concentrations in a typical ICU room with evident high correlation with the distance away from the patient and the level of activity in the ICU rooms.

CONTENTS

ILLUSTRATIONS

TABLES

ABBREVATIONS

AER	Air Exchange Rate
ASHRAE	American Society of Heating, Refrigetating and Air-Conditoining Engineers
CFD	Computational Fluid Dynamics
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
EUCAST	European Committee on Antimicrobial Susceptibility Testting
ICU	Intensive Care Unit
MALTI-TOF	Matrix Assisted Laser Desorption/Ionization – Time Of Flight
MIC	Minimum Inhibitory Concentration
NAAQS	National Ambient Air Quality Standards
PM	Particulate Matter
RH	Relative Humidity
T	Temperature
TBL	Total Bacterial Load
WHO	World Health Organization

CHAPTER I
INTRODUCTION

Air pollution, the fourth leading cause of death worldwide (WHO, 2010), constitutes a global concern that has been subject to extensive research given its direct association with increased morbidity and mortality. Exposure to poor indoor air quality has been linked to major short-term (i.e. eye and throat irritation) as well as long-term health effects (respiratory disease and cancer). Based on cancer-risk alone, indoor air pollution has been ranked among the major environmental problems (Bridger, 2008). Recent efforts focused on assessing the air quality in various indoor environments such as schools, offices and homes, with some work targeting healthcare facilities that contain sources of air pollutants (Table 1) and where the exposure of vulnerable patients to such pollutants can negate the purpose of their hospital visit.

Table 1: Common pollutants in hospitals

		Biological		
Physical	**Chemical**	**Bacteria**	**Fungi**	**Viruses**
TSP[a]	CO[b,d,f]	Staphylococcus[e]	Penicillium[e]	Respiratory syncytial virus[c,e]
PM_{10}[a, e,f]	CO_2[b, d,f]	E.coli[e]	Aspergillus[e]	Influenza[c,e]
$PM_{2.5}$[a, e,f]	SO_2[d]	Streptococcus[e]	Cladosporium[e]	
	NO_x[e]		Alternaria[e]	
	TVOC[b,d,e,f]			

[a] *Nardini et al,2004;* [b] *Erdogan et al, 2009;* [c] *Blachere et al,2009;* [d] *Scheepers et al, 2017;* [e] *Baures et al,2018,* [f] Chamseddine et al, 2015

Particulate matter (PM) is an important IAQ indicator, especially for hospitals, because of their contribution to the transport of bacterial and viral infections (Annesi-Maesano et al., 2007) with many studies reporting elevated PM levels in hospitals (Table 2), due mostly to high indoor-

outdoor correlations and/or to poor performance of HVAC systems (Wang et al, 2006). Similarly, the total bacterial loads (in CFU/m^3) has been measured at different locations within hospitals (Table 3). Reported bactrial concentrations were found to be highly dependent on occupancy levels and the HVAC system (Cabo Verde et al, 2015; Asif et al, 2018). While the ventilation system (natural, mechanical, or mixed) plays a key role in the transport of various pollutants in hospitals (Jung et al, 2015), temperature and relative humidity are known to equally affect the movement, decay and settlement of various pollutants, especially particulate matter and bacteria (Murphy, 2006).

Table 2: Reported particulate matter concentrations in hospitals

	$PM_{2.5}$ (µg/m^3)			**PM_{10} (µg/m^3)**			
Location	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Reference**
Italy	1.6	0.9	0-110	-	-	-	(Nardini et al, 2004)
China	128.1	-	61.7 - 250.	99	-	40.9 - 214.9	(Wang et al, 2006)
USA	19	15	0-100	-	-	-	(Ostro et al., 2009)
Taiwan	1	-	0.1 - 8.4	10	-	0.8-55.6	(Wan et al.,2011)
Portugal	23.4	-	10.5 - 41.9	30.8	-	13 - 58.8	(Slezakova et al., 2012)
Taiwan	14.4	15.9	-	25.2	17.2	-	(Jung et al, 2015)
Netherlands	9.8	-	-	-	-	-	(Scheepers et al., 2017)
France	1.6	-	0 - 45.4	12	-	-	(Baurès et al., 2018)

Table 3:Bacterial concentrations in various hospital units

Hospital Unit	Airborne bacteria concentrations	Findings	Reference
-	240-736 CFU/m^3		(Cabo Verde et al, 2015)
Emergency Services	221-1649.7 CFU/m^3	Highest BL: OPD; Lowest BL: OT1	(Asif et al, 2018)
-	45-150 CFU/plate		(Sudharsanam et al, 2012) [a]
Hospital Ward	1120-168,560 CFU/m^3		(Sudharsanam et al, 2012) [b]
Hospital Ward	3788-191111 CFU/m^3		(Sudharsanam et al, 2012) [c]
Hospital Ward	67-123 CFU/m^3	Lowest BL: General Surgery Highest BL: Transplant Surgery	(Shaw, 2018)
-	87-585 CFU/m^3		(Dai et al, 2015)
-	122-149.7 CFU/m^3		(Pasquarella et al, 2012)
HED **Ambulances** **Offices**	130-4200 CFU/m^3 130-1400 CFU/m^3 42-5000 CFU/m^3		(Bielawska-Drózd et al, 2018)

[a] Passive Sampling; [b] Impingement Sampling; [c] Filter Sampling

CFU: Colony forming unit, BL: Bacterial Level, OPD: Out-Patient Department, HED: Hospital Emergency Department. BL: Bacterial Load

In this study, we present a first attempt at examining the spatial variation of airborne bacterial concentrations in ICU rooms and evaluate their variability as a function of particle size to provide an understanding of the factors affecting bacterial loads in ICUs. For this purpose, we monitored particulate matter and airborne bacterial levels with their corresponding diameter sizes in ICU rooms. Several physical parameters were concurrently measured, and multivariate regression models were developed using correlations between pollutant levels and physical parameters.

CHAPTER II
MATERIALS AND METHODS

A. Study Design

The monitoring program was implemented across 10 ICU rooms during night hours (7:00 pm-4:00 am) at the American University of Beirut Medical Center (AUBMC) after 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 patients approached, accepted to take part of this study.

The monitored parameters included the Total Bacterial Load (TBL), the Gram-negative bacterial load, Particulate Matter (PM_{10} and $PM_{2.5}$) concentrations, Temperature (T), Relative Humidity (RH), and the distribution of bacteria by their diameter size. Additionally, the occupancy, level of activity, and room volume were recorded.

A factory calibrated TSI DustTrak™ II Aerosol Monitor (Model 8532, TSI Corporation, Shoreview, USA) was used to measure PM_{10} and $PM_{2.5}$ concentrations and a Langan L76x Air Quality Analyzer was used to measure for RH and T. A 6-stage viable impactor sampling system was used for the fractionation of bacteria to simulate the various stages of the human respiratory track. The diameters 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 conducted using the Six Stage Microbial Andersen Cascade Impactor (TISH Environmental Model TE-10-800) 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 at the beginning of every sampling round using a rotameter airflow meter with a capacity of 2

ft^3/min. In each room, six samples were collected at the breathing level of 1.5 meters for a duration of 20 minutes. Two simultaneous samples were collected at 0.5 and 1.5 meters away from the patient and tested for Total Bacteria load (TBL). Subsequently, another two simultaneous samples at 1 and 2 meters away from the patient were also recorded. Finally, two simultaneous samples at 0.5 and 1.5 meters were taken and tested for Gram-negative Bacteria. Two additional samples at distance of 0.5 and 1.5 meters 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, while the concentrations of TBL was 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 allow 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 hours 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 minutes as expressed below.

$$TBL = \frac{\mathrm{C} \times 1000}{V}$$

Where $V = 566\ L$ is the volume of air sampled in 20 minutes, C is the bacterial count, and TBL is the Total Bacterial Load expressed in number of Colony Forming Units (CFU) per 1 m^3 of air.

During the sampling process in a typical room (Figure 1), the door was always kept closed. Also, the pumps were activated from outside the rooms 10 minutes after they were installed in their sampling locations so as 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, with the exception of two rooms (Room ID 3 and 6 in Figure 2), where a private nurse was present at all times. The nurse was asked to remain seated during the sampling period. As for the regular hospital nurses' trips, most were short (< 1 minute).

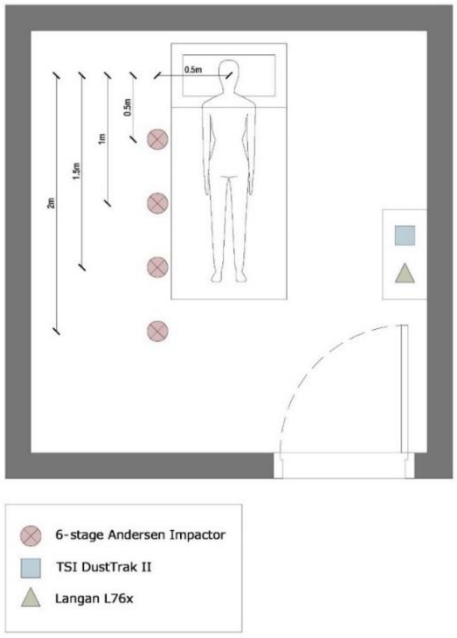

Figure 1: Top view of a typical ICU room

B. Statistical Analysis

Correlations, ANOVA and multiple regression were used to assess the importance of several factors in predicting bacterial concentration values in ICU rooms. The average indoor $PM_{2.5}$ and PM_{10} levels were compared with IAQ guidelines (WHO, 2010). The 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 studio team, 2015).

C. Isolates Collection and Broth Microdilution

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. 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 microdillution against ertapenem, meropenem, imipenem, gentamicin, ciprofloxacin, cefepime, vancomycin, and dalfopristin 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 $5x10^6$ CFU/mL. From the latter, 10 µL were added to each well between columns 1 and 11, leading to a final bacterial concentration of $5x10^5$

CFU/mL. Each plate was then incubated at 37 °C for 18-24 hours, 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 12,000g 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 °C. 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).

CHAPTER III
RESULTS AND DISCUSSION

A. Thermal comfort and PM

The measured temperature (T) in the ICU rooms ranged from 22.0 to 23.5 °C (mean=22.5°C, SD=0.46), while the relative humidity (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 strict ventilation standards and the 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 fell within the range reported in other studies (Ostro et al., 2009; Slezakovaet al., 2012) with several rooms exceeding international guidelines for 24-hr PM exposure (Figure 2) (WHO, 2010). 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 actually $PM_{2.5}$ (mean of $PM_{2.5}/PM_{10}$ ratio = 0.90, SD = 0.1). Surprisingly, a low correlation (Table 4) was observed between PM concentrations and level of activity in each room (number of trips and occupancy rate). This low correlation could be due to the fact that most PM in the ICU rooms was in the form of fine particles that tend to remain permanently suspended and slightly affected by resuspension (Hospodsky et al, 2012).

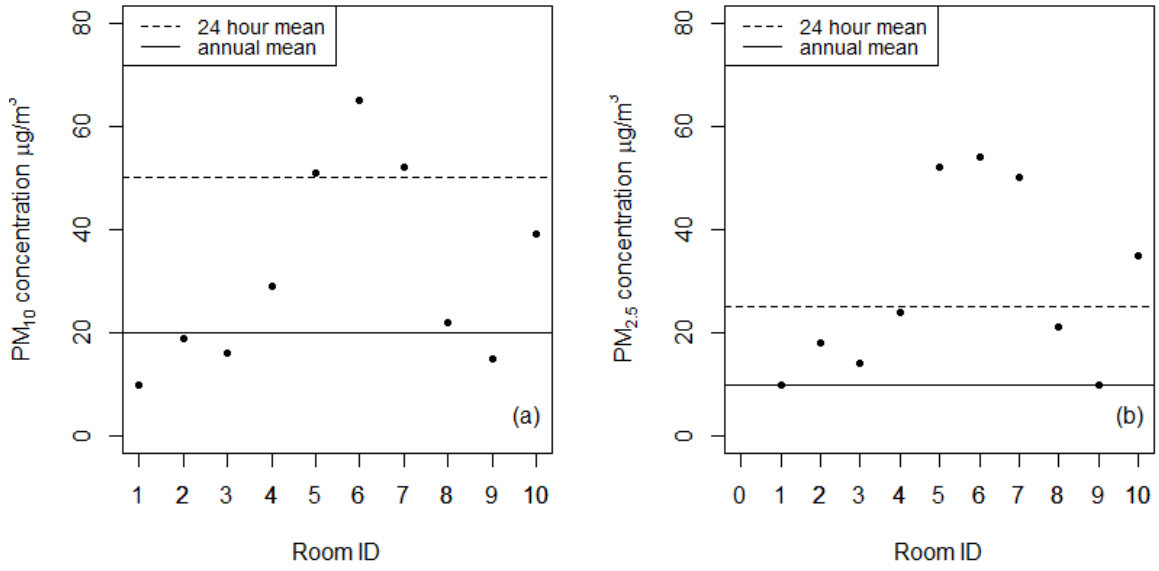

Figure 2: In-patient PM concentration compared to WHO guidelines (WHO,2010)

Table 4: Correlation of measured PM concentrations with the level of activity

	PM_{10}	**$PM_{2.5}$**
Number of trips	r[a]=0.30	r=0.26
	p=0.06[b]	p=0.11
Occupancy	r=0.13	r=0.1
	p=0.43	p=0.51

[a] Pearson's correlation coefficient
[b] Significant to the 10% level

B. Total Bacterial Load

The TBL concentrations (Appendix A) ranged from 20.4 to 134.3 CFU/m^3 (mean= 66.43, SD= 35.20) with no significant correlations between TBL and the measured T and RH because the latter remained relatively constant across all ICU rooms (Table 5). 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) (Figure 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 the 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 3 or more trips occurred was significantly higher than the rest (mean= 117.3 CFU/m^3, p-value< 0.05). Similarly and as expected, the occupancy level was found to affect the measured bacterial concentrations in the air, since additional occupants can be both bacteria sources and their activity may also 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 level of activity in the ward was highly correlated with the airborne concentrations of bacteria, while the presence of sedentary visitors was not. In this study, since most occupants were relatively static, their contribution to the resuspension of bacteria should be low. 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 31.68 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 meters (Figure 3). We believe that 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. It is also important to note that TBL and PM levels were not strongly correlated, which indicates that the two have different sources.

Table 5:Total Bacterial Load correlations with measured parameters

	TBL
Temp	-0.31 [a]
RH	-0.15
PM_{10}	0.22
$PM_{2.5}$	0.21
Trips	0.86 [b]
Occupancy	0.61 [b]
Distance	-0.12

[a] Significant to the 10% level
[b] Significant to the 1% level

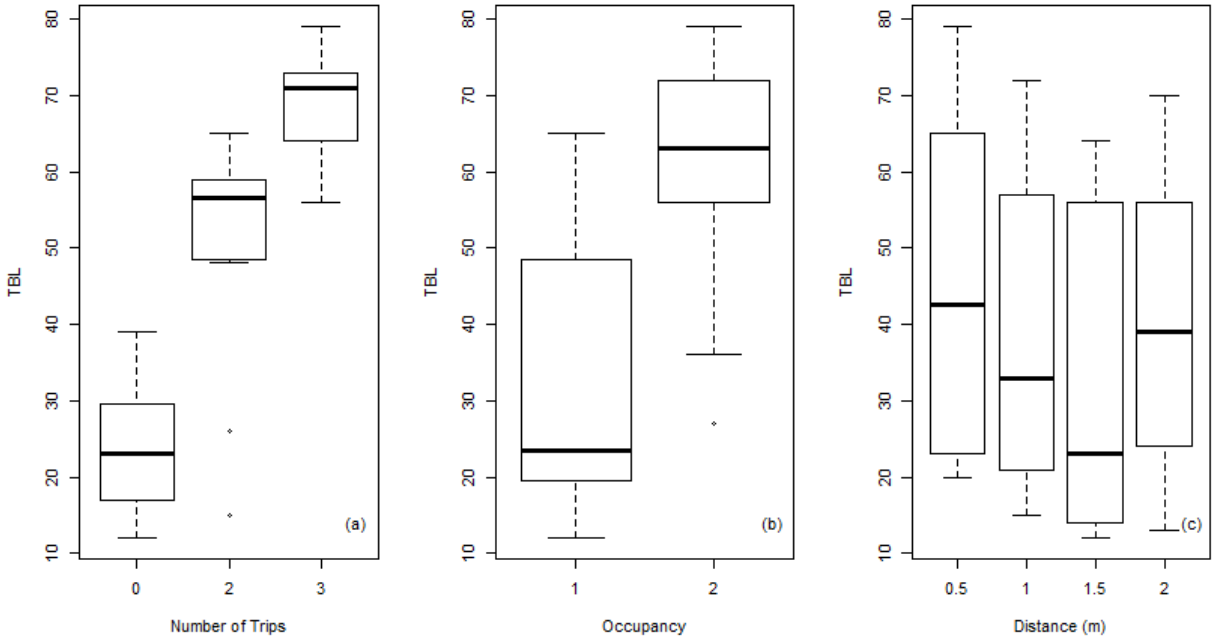

Figure 3: TBL as a function of (a) Number of trips, (b) Occupancy, and (c) Distance away from patient in meters

C. Bacterial Load Contribution by size

Examining the bacteria load contributions (BLC) for each size (Appendix A), 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 the Holm's correction showed that the mean BLC for the bacterial sizes less than 0.65 micron (mean BLC = 5%) were statistically lower than all other bacterial sizes (p-value < 0.05). The mean BLC for bacteria with sizes between 0.65 and 1.1 microns (mean BLC = 33%) were significantly higher than the rest of the bacterial size groups (p-value< 0.05) (Figures 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.

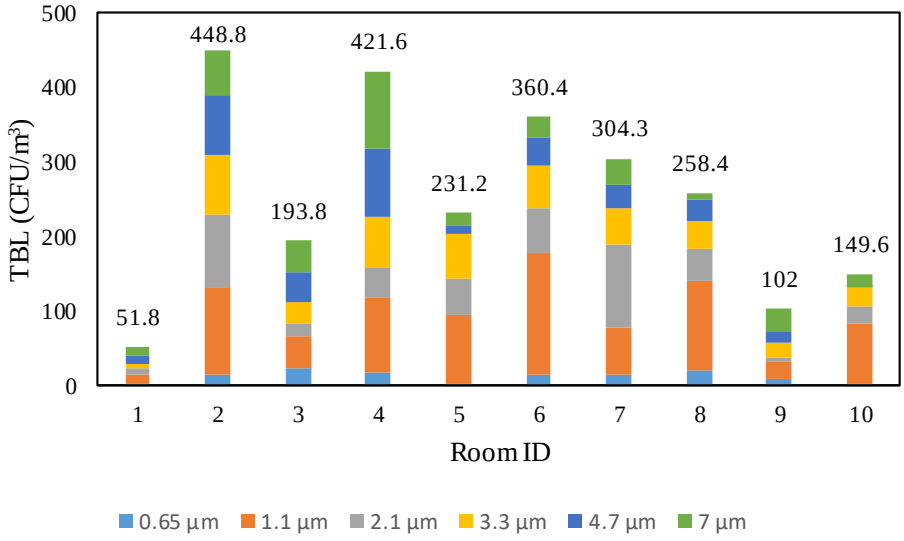

Figure 4: Bacteria concentration by size and room

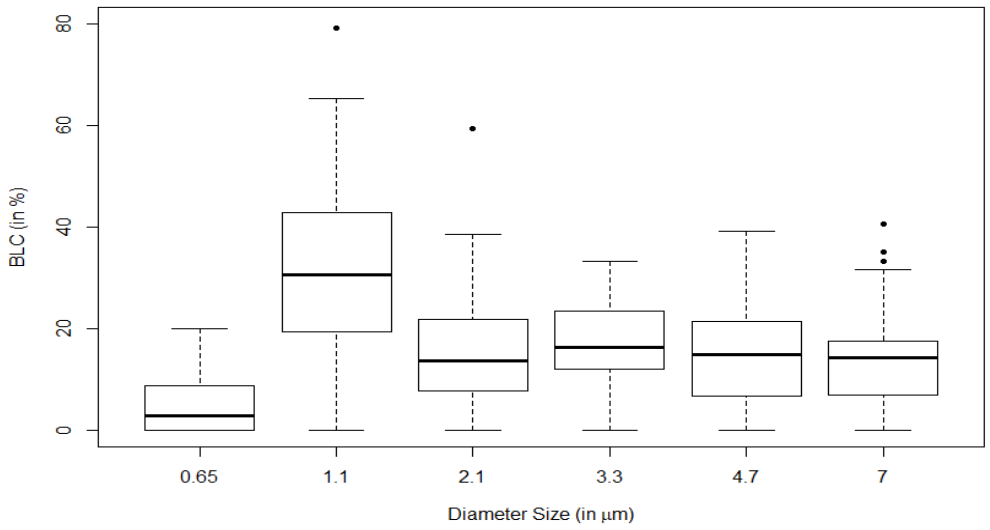

Figure 5: Distribution of bacteria by diameter size

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 µm and 4.7 µm, and the large category for those with diameters > 4.7 µm. The correlation between the concentrations and distance away from the patient showed no correlation between distance and the small particles (r=0). A weak negative correlation was found with the bacteria in the larger bacterial size group (Table 6). These results indicate that the heavier the bacteria, the higher the probability that it will settle with distance. On the other hand, and for the small sized and light bacteria, they appear to be well-mixed in the room irrespective of distance. As for the correlation between the bacteria 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. Strong positive correlations were found between the number of trips on one hand and the medium and large sized particles 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.

Table 6:Correlations of Bacterial Concentrations with physical parameters

	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

D. TBL regression model

A regression model was developed to predict the measured TBL levels as a function of the room characteristics and occupancy levels (Table 7). The model showed that distance away from the patient and its squared value (to account for the increase in concentrations at 2 meters) 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). 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 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 meters 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%) (Figure 4). 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.

Table 7:Regression Model Parameters for Total Bacterial Load (TBL)

Variable	Unit	Estimate	t-statistic	P-value
Intercept	CFU/m^3	57.1 [c]	3.298	0.00234
Distance (D)	m	-66.0 [b]	-2.41	0.02
Distance squared (D^2)	m^2	25.79 [b]	2.37	0.02
Number of trips (T)		21.49 [c]	8.05	2.74×10^{-9}
Occupancy (O)		15.60 [b]	2.11	0.04
		R^2 = 0.774		
TBL = 57.120 – 66.003 D + 25.875 D^2 + 21.487 T + 15.597 O				

[a] Significant to the 10% level;
[b] Significant to the 5% level;
[c] Significant to the 1% level

E. Microbiological analysis 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 Matrix Assisted Laser Desorption/Ionization – Time Of Flight (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 8).

Table 8: 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*

Broth microdilution 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 cefepime, their breakpoints were not specified according to the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testting (EUCAST) guidelines (Table 9). 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 threat, if one of these isolates were acquired by a patient from a healthcare worker. This is why healthcare professionals must take good care of their skin hygiene to halt the possible transmission of resistant skin flora to their patients. Furthermore, we did not isolate any multi-drug resistant Gram-negative bacilli, which is a sign that aerosolized pathogens in the ICU are probabily absent.

Table 9: MIC results of 10 isolates against 8 different 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)	1 (S)	<0.125 (S)
3	1	16	0.5	8	<0.125 (S)	0.25 (S)	4 (S)	<0.125 (S)
4	>128	8	0.5	4	>128 (R)	0.5 (S)	4 (S)	<0.125 (S)
5	4	128	16	128	0.25 (S)	<0.125 (S)	2 (S)	<0.125 (S)
6	8	>128	16	128	<0.125 (S)	0.25 (S)	2 (S)	<0.125 (S)
7	32	>128	1	>128	128 (R)	8 (R)	2 (S)	<0.125 (S)
8	4	16	<0.125	16	8 (I)	1 (S)	0.5 (S)	<0.125 (S)
9	16	>128	32	>128	128 (R)	8 (R)	2 (S)	<0.125 (S)
10	4	32	<0.125	4	<0.125 (S)	4 (R)	2 (S)	<0.125 (S)
11	32	>128	128	>128	128 (R)	128 (R)	2 (S)	<0.125 (S)

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.

CHAPTER IV

CONCLUSION

Indoor air quality was examined in ICUs through monitoring of $PM_{2.5}$, PM_{10}, T, RH and TBL at several distances from patients in parallel with the assessment of the level of activity through quantifying room occupancy and the number of nurses' trips inside the room. The latter was found to be an important potential contributor to airborne bacterial concentrations along with the distance from the source. A statistical analysis and a linear regression model were built to predict the concentrations at different points in a typical ICU room. Several antibiotic-resitant bacterial species were collected and identified. The results raise concerns about IAQ in ICUs requiring mitigation measures (Table 10) that can provide thermal comfort and reduce concentrations of airborne contaminants.

Table 20:Mitigation meausres to control air quality in ICUs

Parameter	Mitigation Measure
Bioaerosols and Particulate Matter	• Regular surface cleaning for the ICU rooms to remove settled PM and bioaerosols • Reduction in occupancy inside ICU rooms • Reduction in the nurses' trips inside the rooms by performing as many possible tasks in one entry • Reduction of "high resuspension" activities (curtain or sheets movement…) • Enforcement to wear of gloves and gowns on all visitors whenever it is required, to avoid the introduction of new bioaerosols from the outside environment • UV disinfection after patient dismissal, especially for rooms where patient had a strong bacterial infection (*Clostridium difficile, Acinetobacter*…)
Thermal Comfort	• Maintenance of T and RH levels as per ASHRAE standards
Ventilation	• Regular replacement of the system's filters • Regular measurement of the air inflow • Maintenance of lower air pressure inside rooms to reduce aerosols transport • Application and monitoring of ASHRAE standards for ventilation (Air Exchange Rate, humidity requirements, pressurization…)

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 IAQ standards although benchmark guidelines have been reported (Capolongo and Gola, 2017).

REFERENCES

- ANNESI-MAESANO, I.F., F. Kunzli, N. Brunekref, B. 2007. Particulate matter, science and EU policy. *European Respiratory Journal.* **29** , pp.428-431.
- ASIF, A., Zeeshan, M., Hashmi, I., Zahid, U., Bhatti, M.F. 2018. Microbial quality assessment of indoor air in a large hospital building during winter and spring seasons. *Building and Environment*. **135**, pp.68–73.
- BAURES, E., Blanchard, O., Mercier, F., Surget, E., le Cann, P., Rivier, A., Gangneux, J. and Florentin, A. 2018. Indoor air quality in two French hospitals: Measurement of chemical and microbiological contaminants. *Science of The Total Environment*. **642**, pp.168-179.
- BIELAWSKA-DROZD, A., Cieślik, P., Bohacz, J., Korniłłowicz-Kowalska, T., Żakowska, D., Bartoszcze, M., Wlizło-Skowronek, B., Winnicka, I., Brytan, M., Kubiak, L., Skopińska-Różewska, E., and Kocik, J. 2018. Microbiological analysis of bioaerosols collected from Hospital Emergency Departments and ambulances. *Ann Agric Environ Med.*, **25**(2),pp.274-279. https://doi.org/10.26444/aaem/80711
- BLACHERE, F.M., Lindsley, W.G., Pearce, T.A., Anderson, S.E., Fisher, M., Khakoo, R., Meade, B.J., Lander, O., Davis, S., Thewlis, R.E., Celik,I., Chen, B.T., Beezhold, D.H. 2009. Measurement of Airborne Influenza Virus in a Hospital Emergency Departmet. *Clinical Infectious Diseases.* **48**, pp. 438-440.
- BRIDGER, C. 2008. National Public Health Week: The Health Impacts of Indoor Air Quality. *EPA.* (Accessed: 12 February 2020)
- CABO VERDE, S., Almeida, S.M., Matos, J., Guerreiro, D., Meneses, M., Faria, T., Botelho, D., Santos, M., Viegas, C. 2015. Microbiological assessment of indoor air quality at different hospital sites. *Research in Microbiology.* **166**, pp.557–563.
- CAPOLONGO, S., Settimo, G., & Gola, M..(2017) *Indoor Air Quality in Healthcare Facilities*. *E-book* library [online]. Available at: https://link.springer.com/book/10.1007%2F978-3-319-49160-8 (Accessed: 15 February 2020)
- CHAMSEDDINE, A., El-Fadel, M. 2015. Exposure to air pollutants in hospitals: indoor-outdoor correlations. Southampton: W I T Press.
- CHEN, Q.a.H., L. 2009. The Effects of Human Activities on Exposure to Particulate Matter and Bioaerosols in Residential Homes. *Environmental Science & Technology*. **43**(13), pp.4641-4646.
- CLAUS, M.2015. Particle size distribution of airborne microorganisms in the environment – A review. *Landbauforsch Appl Agric Forestry Res*, **65**(2),pp.77-100.
- DAI, C., Zhang, Y., Ma, X., Yin, M., Zheng, H., Gu, X., Xie, S., Jia, H., Zhang, L. and Zhang, W. 2015. Real-time measurements of airborne biologic particles using fluorescent particle counter to evaluate microbial contamination: Results of a comparative study in an operating theater. *American journal of infection control*. **43**(1), pp. 78-81.

- ERDOGAN, M., Yurtseven, E., Erginoz, E., Vehid, S., Koksal, S., & Yuceokur, A. 2009. Total volatile organic compounds (TVOC), carbon monoxide (CO), carbon dioxide (CO2) concentrations in the hospital building of a medical faculty in Istanbul, *Turkey*. . *Nobel Medicus*. **18**, pp.66 – 72.
- FITZPATRICK, F., Humphreys, H., Smyth, E., Kennedy, C. A., & O'Gara, J. P. 2002. Environmental regulation of biofilm formation in intensive care unit isolates of Staphylococcus epidermidis. *Journal of Hospital Infection*. **52**(3), pp.212-218
- GARZA-GONZALEZ, E., Morfin-Otero, R., Martínez-Vázquez, M. A., Gonzalez-Diaz, E., González-Santiago, O., & Rodríguez-Noriega, E. 2011. Microbiological and molecular characterization of human clinical isolates of Staphylococcus cohnii, Staphylococcus hominis, and Staphylococcus sciuri. *Scandinavian journal of infectious diseases*. **43**(11-12), pp.930-936.
- HATHWAY, Abigail & Noakes, Catherine & Sleigh, Andrew & Fletcher, L.a. 2011. CFD simulation of airborne pathogen transport due to human activities. *Building and Environment*. **46,** p.2500-2511.
- HOSPODSKY, D., Qian, J., Nazaroff, W., Yamamoto, N., Bibby, K., Rismani-Yazdi, H. and Peccia, J. 2012. Human Occupancy as a Source of Indoor Airborne Bacteria. *PLoS ONE*, **7**(4), p.e34867.
- JUNG, C. C., Wu, P. C., Tseng, C. H., & Su, H. J. 2015. Indoor air quality varies with ventilation types and working areas in hospitals. *Building and Environment*. **85**, pp.190 – 195.
- KIM, K.Y., Kim, Y.S., Kim, D. 2010. Distribution Characteristics of Airborne Bacteria and Fungi in the General Hospitals of Korea. *Industrial Health*. **48**, pp.236–243.
- MURPHY J. 2006. Temperature and humidity control in surgery rooms. *Health Care HVAC ASHRAE Journal*. **48**, pp.18 – 25.
- NARDINI S, C.R., Invernizzi G, Ruprecht A, Boffi R., Formentini S. 2004. *Monaldi Archive for Chest Diseases*. **61**(3), pp.183 – 192.
- OSTRO, B., Roth, L., Malig, B., & Marty, M.2009. The Effects of Fine Particle Components on Respiratory Hospital Admissions in Children. *Environment Health Perspective*. **117**(3), pp.475 – 480.
- PASQUARELLA, C., Vitali, P., Saccani, E., Manotti, P., Boccuni, C., Ugolotti, M., Signorelli, C., Mariotti, F., Sansebastiano, G. and Albertini, R. 2012. Microbial air monitoring in operating theatres: experience at the University Hospital of Parma. *Journal of Hospital Infection*. **81**(1), pp.50-57.
- R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- SCHEEPERS, P., Van Wel, L., Beckmann, G. and Anzion, R. 2017. Chemical Characterization of the Indoor Air Quality of a University Hospital: Penetration of Outdoor Air Pollutants. *International Journal of Environmental Research and Public Health*. **14**(5), p.497.

- SHAW, L. F., Chen, I. H., Chen, C. S., Wu, H. H., Lai, L. S., Chen, Y. Y., & Der Wang, F. 2018. Factors influencing microbial colonies in the air of operating rooms. *BMC infectious diseases*. **18**(1), p.4.
- SLEZAKOVA, K., da Conceição Alvim-Ferraz, M., & do Carmo Pereira, M. 2012. Elemental characterization of indoor breathable particles at a Portuguese urban hospital. *Journal of Toxicology and Environmental Health, Part A*. **75**(13-15), pp.909-919.
- SUDHARSANAM, S., Swaminathan, S., Ramalingam, A., Thangavel, G., Annamalai, R., Steinberg, R., ... & Srikanth, P. 2012. Characterization of indoor bioaerosols from a hospital ward in a tropical setting. *African health sciences*. **12**(2),pp. 217-225.
- WAN, G. H., Chung, F. F., & Tang, C. S. 2011. Long term surveillance of air quality in medical center operating rooms. *American Journal of Infection Control*. **39**(4), pp.302 – 308.
- WANG, X., Bi, X., Sheng, G. and Fu, J.2006. Hospital indoor PM10/PM2.5 and associated trace elements in Guangzhou, China. *Science of The Total Environment*. **366**(1), pp.124-135.
- WHO, R. 2010. WHO guidelines for indoor air quality: selected pollutants. *WHO Regional Office for Europe*. pp.289-346.

APPENDIX A

Size (µm)	Distance (m)	Trips (Count)	Occupancy (Count)	Volume (m^3)	Concentration (CFU/m^3)	TBL (CFU/m^3)
7	0.5	0	1	43.2	11.9	39.1
4.7					8.5	
3.3					5.1	
2.1					5.1	
1.1					5.1	
0.65					3.4	
7	1.5	0	1	43.2	3.4	23.8
4.7					5.1	
3.3					0	
2.1					6.8	
1.1					6.8	
0.65					1.7	
7	0.5	2	2	76.95	10.2	100.3
4.7					23.8	
3.3					8.5	
2.1					34	
1.1					22.1	
0.65					1.7	
7	1	4	2	76.95	18.7	122.4
4.7					18.7	
3.3					25.5	
2.1					23.8	
1.1					23.8	
0.65					11.9	
7	1.5	2	2	76.95	13.6	105.4
4.7					17	
3.3					22.1	
2.1					13.6	
1.1					39.1	
0.65					0	
7	2	4	2	76.95	17	119
4.7					20.4	
3.3					23.8	
2.1					25.5	
1.1					30.6	
0.65					1.7	
7	0.5	0	1	40.5	5.1	39.1
4.7					15.3	
3.3					10.2	
2.1					3.4	
1.1					3.4	
0.65					1.7	
7	1	0	1	40.5	5.1	35.7
4.7					8.5	
3.3					6.8	
2.1					5.1	
1.1					6.8	
0.65					3.4	
7	1.5	0	1	40.5	8.5	64.6
4.7					10.2	
3.3					8.5	
2.1					1.7	
1.1					27.2	
0.65					8.5	
7	2	0	1	40.5	22.1	54.4
4.7					8.5	
3.3					3.4	
2.1					6.8	

1.1 0.65					5.1 8.5	
7 4.7 3.3 2.1 1.1 0.65	0.5	3	2	76.95	42.5 37.4 27.2 8.5 17 1.7	134.3
7 4.7 3.3 2.1 1.1 0.65	1	2	1	76.95	17 34 6.8 10.2 17 11.9	96.9
7 4.7 3.3 2.1 1.1 0.65	1.5	3	2	76.95	28.9 8.5 20.4 3.4 32.3 1.7	95.2
7 4.7 3.3 2.1 1.1 0.65	2	2	1	76.95	15.3 11.9 13.6 18.7 34 1.7	95.2
7 4.7 3.3 2.1 1.1 0.65	0.5	2	1	44.55	8.5 3.4 27.2 18.7 25.5 0	83.3
7 4.7 3.3 2.1 1.1 0.65	1	0	1	44.55	5.1 1.7 6.8 18.7 22.1 1.7	56.1
7 4.7 3.3 2.1 1.1 0.65	1.5	2	1	44.55	0 5.1 6.8 6.8 6.8 0	25.5
7 4.7 3.3 2.1 1.1 0.65	2	0	1	44.55	3.4 1.7 18.7 5.1 35.7 1.7	66.3
7 4.7 3.3 2.1 1.1 0.65	0.5	3	2	76.95	13.6 10.2 17 18.7 59.5 5.1	124.1
7 4.7 3.3 2.1 1.1 0.65	1	2	1	76.95	1.7 3.4 6.8 3.4 28.9 0	44.2
7 4.7	1.5	3	2	76.95	1.7 8.5	108.8

3.3 2.1 1.1 0.65					25.5 23.8 45.9 3.4	
7 4.7 3.3 2.1 1.1 0.65	2	2	1	76.95	11.9 13.6 10.2 13.6 28.9 5.1	83.3
7 4.7 3.3 2.1 1.1 0.65	0.5	0	2	40.5	10.2 6.8 20.4 10.2 11.9 1.7	61.2
7 4.7 3.3 2.1 1.1 0.65	1	2	1	40.5	6.8 10.2 6.8 37.4 23.8 11.9	96.9
7 4.7 3.3 2.1 1.1 0.65	1.5	0	2	40.5	8.5 10.2 11.9 3.4 11.9 0	45.9
7 4.7 3.3 2.1 1.1 0.65	2	2	1	40.5	8.5 5.1 10.2 59.5 17 0	100.3
7 4.7 3.3 2.1 1.1 0.65	0.5	2	1	43.2	0 8.5 17 34 42.5 8.5	110.5
7 4.7 3.3 2.1 1.1 0.65	1	2	1	43.2	3.4 11.9 11.9 3.4 39.1 11.9	81.6
7 4.7 3.3 2.1 1.1 0.65	1.5	0	1	43.2	3.4 3.4 6.8 1.7 6.8 0	22.1
7 4.7 3.3 2.1 1.1 0.65	2	0	1	43.2	1.7 6.8 0 3.4 28.9 0	40.8
7 4.7 3.3 2.1 1.1 0.65	0.5	0	1	45.9	11.9 8.5 6.8 0 6.8 0	34

7 4.7 3.3 2.1 1.1 0.65	1	0	1	45.9	8.5 1.7 3.4 3.4 3.4 5.1	25.5
7 4.7 3.3 2.1 1.1 0.65	1.5	0	1	45.9	6.8 0 6.8 1.7 1.7 3.4	20.4
7 4.7 3.3 2.1 1.1 0.65	2	0	1	45.9	3.4 5.1 1.7 1.7 10.2 0	22.1
7 4.7 3.3 2.1 1.1 0.65	0.5	0	1	36.45	1.7 1.7 10.2 1.7 23.8 0	39.1
7 4.7 3.3 2.1 1.1 0.65	1	0	1	36.45	8.5 0 1.7 11.9 11.9 1.7	35.7
7 4.7 3.3 2.1 1.1 0.65	1.5	0	1	36.45	0 0 5.1 5.1 18.7 0	28.9
7 4.7 3.3 2.1 1.1 0.65	2	0	1	36.45	6.8 0 6.8 1.7 22.1 1.7	39.1