Factors that affect the relationship between microorganisms in the air and on surfaces in hospital environments

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Intellectual Property and Publication Statements

The candidate confirms that the work submitted is his own, and that appropriate credit has been given where reference has been made to the work of others.

The work in **Chapter 3** of the thesis is based on this publication as follows:

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Abstract

Hospital acquired infections (HAIs) incur mortalities and high costs for treatment including the ongoing outbreak of the COVID-19 pandemic as a result of contaminated air and surfaces. Understanding the relationship between microorganisms in the air and on surfaces and assessing how hospital environment factors affect this relationship is essential to mitigate the infection risk.

A systematic review and meta-analysis showed that there were positive correlations between the airborne bacteria measured as aerobic colony count (ACC) and indoor air quality (IAQ) parameters in the hospital. Also, previous mathematical approaches to correlating air and surface microorganisms were identified and evaluated against published data.

Environmental sampling was carried out to explore the transient relationships between air and surface bioburden in a hospital. The average concentration of ACC in air was 196±103 cfu.m^{-3}, while our multi-regression model recommended a maximum of 118 cfu.m^{-3}. High touch surfaces in 1-bed, 4-bed, and 10-bed rooms, found 100%, 84%, and 73% surface deposition samples within an accepted level of concentration (<2.5 cfu.cm^{-2}). The loss rate due to deposition onto surfaces (λ_d) was 7.2±2.88h^{-1} for ACC and 4.32±2.88h^{-1} for *Staphylococcus spp.*

The effect of ventilation and spatial location on (λ_d) was investigated by developing and validating a novel Automated Multiplate Passive Air Sampling (AMPAS) device. Increasing the ventilation rate from 3 to 6 ACH results in a reduction of *Staphylococcus aureus* load in air and on surfaces by 45%±10% and 44%±32%, respectively and λ_d was 1.38±0.48h^{-1} in the chamber.

The relationship between microorganisms in the air and on surfaces is a significant parameter in an infection risk model. It can be represented by λ_d, however this is complex to determine in a dynamic environment. The findings support the importance of controlling the ventilation and the environmental parameters to mitigate both air and surface infection risks in the hospital environment.

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Abbreviations

ACC	**A**erobic **C**olony **C**ount
ACH	**A**ir **C**hanges per **H**our
AMPAS	**A**utomated **M**ultiplate **P**assive **A**ir **S**ampling
ANOVA	**AN**alysis **O**f **VA**riance
CDC	**C**entre for **D**isease **C**ontrol and Prevention
C. difficile	***C****lostridium* ***difficile***
CFD	**C**omputational **F**luid **D**ynamics
CI	**C**onfidence **I**nterval
CO_2	**C**arbon **D**ioxide
COVID-19	**CO**rona**Vi**rus **D**isease 2019
DAG	**D**irected **A**cyclic **G**raph
E. Coli	***E****scherichia* ***coli***
EU-GMP:	**E**uropean **U**nion - **G**ood **M**anufacturing **P**ractices
HAIs	**H**ospital **A**cquired **I**nfection**s**
HCAIs	**H**ealthcare-**A**ssociated **I**nfection**s**
HCW	**H**ealth**C**are **W**orker
HECOIRA	**H**ospital **E**nvironment **C**ontrol, **O**ptimisation and **I**nfection **R**isk **A**ssessment
HEPA	**H**igh-**E**fficiency **P**articulate **A**bsorbing
IAQ	**I**ndoor **A**ir **Q**uality
ICU	**I**ntensive **C**are **U**nit
MRSA	**M**ethicillin-**R**esistant ***S****taphylococcus* ***a****ureus*
MSA	**M**annitol **S**alt **A**gar
NHS	**N**ational **H**ealth **S**ervice
NS	**N**on-**S**ignificance
PM	**P**articulate **M**atter
PRISMA	**P**referred **R**eporting **I**tems for **S**ystematic Reviews and **M**eta-**A**nalyses

QMRA	**Q**uantitative **M**icrobial **R**isk **A**ssessment
RH	**R**elative **H**umidity
ROC	**R**eceiver **O**perating characteristics **C**urves
SAR	**S**urface **A**ir **R**atio
SARS-CoV-2	**S**evere **A**cute **R**espiratory **S**yndrome **CO**rona**V**irus-**2**
S. aureus	***S****taphylococcus* ***aureus***
Sig	**Sig**nificance
TB	**T**uberculosis
TF	**T**otal **F**ungi
TSA	**T**ryptone **S**oya **A**gar
UV	**U**ltraviolet **C**
VRE	**V**ancomycin-**R**esistant **E**nterococci
WHO	**W**orld **H**ealth **O**rganization

Symbols

a	**S**urface **A**rea of a plate	cm^{-2}
$\boldsymbol{A_c}$	**A**rea of a **C**eiling	m^2
$\boldsymbol{A_f}$	**A**rea of a **F**loor	m^2
$\boldsymbol{A_w}$	**A**rea of a **W**all	m^2
$\boldsymbol{C}$	**C**oncentration of airborne microorganisms	$cfu.m^{-3}$
$\boldsymbol{C_s}$	Deposition rate of airborne microorganisms on **S**urfaces	$cfu.m^{-2}.h^{-1}$
$\boldsymbol{C_{sf}}$	Deposited microorganisms' **C**oncentration on a **F**loor	$cfu.m^{-2}.h^{-1}$
$\boldsymbol{C_{sc}}$	Deposited microorganisms' **C**oncentration on a **C**elling	$cfu.m^{-2}.h^{-1}$
$\boldsymbol{C_{sw}}$	Deposited microorganisms' **C**oncentration on a **W**all	$cfu.m^{-2}.h^{-1}$
$\boldsymbol{F_D}$	**D**rag **F**orce	$kg.m.s^{-2}$
$\boldsymbol{F_g}$	**G**ravity **F**orc	$kg.m.s^{-2}$
$\boldsymbol{g}$	**G**ravitational field strength	$m.s^{-2}$
$\boldsymbol{q}$	Constant microorganism's generation rate in a room space	$cfu.s^{-1}$
$\boldsymbol{i}$	Number of samples	-
$\boldsymbol{m_{3,l}}$	**M**ean of deposited microorganism load in specific **L**ocation at **3** ACH	$cfu.m^{-2}.h^{-1}$
$\boldsymbol{N}$	Air-change rate per hour	h^{-1}
$\boldsymbol{n_{i,6,l}}$	Single data value of deposited microorganism load at the same location l for sample i	$cfu.m^{-2}.h^{-1}$
$\boldsymbol{P_f}$	**D**ensity of the **F**luid	$kg.m^{-3}$
$\boldsymbol{P_p}$	**D**ensity of the **P**article	$kg.m^{-3}$
$\boldsymbol{Q}$	Ventilation rate	$l.s^{-1}$

$\boldsymbol{R}$	**R**adius	m
$\boldsymbol{R_l}$	Percentage of **R**eduction	-
$\boldsymbol{V}$	**V**olume of a room	m^3
$\boldsymbol{v}$	**V**elocity	$m.s^{-2}$
$\boldsymbol{\beta}$	Efficiency of generation rate	-
$\boldsymbol{\beta_c}$	Percentages deposited microorganisms' concentration on the ceiling compared to floor	-
$\boldsymbol{\beta_w}$	Percentages deposited microorganisms' concentration on the wall compared to floor	-
$\boldsymbol{\gamma}$	Loss rate due to decay	h^{-1}
$\boldsymbol{\lambda_d}$	Loss rate due to **D**eposition onto all room surfaces per hour	h^{-1}
$\boldsymbol{\lambda_{d.c}}$	Loss rate due to **D**eposition onto **C**elling per hour	h^{-1}
$\boldsymbol{\lambda_{d.f}}$	Loss rate due to **D**eposition onto **F**loor per hour	h^{-1}
$\boldsymbol{\lambda_{d.w}}$	Loss rate due to **D**eposition onto **W**all per hour	h^{-1}
$\boldsymbol{\lambda_T}$	**T**otal loss rate	h^{-1}
$\boldsymbol{\lambda_v}$	Loss rate due to ventilation	h^{-1}

1 Introduction

1.1 Healthcare-Associated Infections

Hospital acquired infections (HAIs) or nosocomial infections are infections that occur for patients, staff or visitors who go into a healthcare setting (such as a hospital) even though they had not had it before being admitted (Wade et al., 2021). HAIs can be mortal, and they are a globally significant problem that incurs high costs for treatment (Setlhare et al., 2014; Dancer, 2014; Guest et al., 2020).

HAIs continue to be a major concern for the National Health Service (NHS) due to the continued rise of antibiotic resistance amongst microorganisms, which leads to increased death rates and treatment costs. In 2017, about 22800 mortalities resulted from HAIs, and the treatment of patients with HAIs in England alone cost £2.1 billion (Guest et al., 2020). The nosocomial transmission is also important for viral infections and has been significant with the ongoing outbreak of the severe acute respiratory syndrome coronavirus-2/coronavirus disease 2019 (SARS-CoV-2/COVID-19). In the first wave, around 1% of all tested cases in the UK were estimated to be nosocomial and the nosocomial infection rate was around 20-25% (PHE and LSHTM, 2021). Across the UK there have been 159,716 mortalities and 7,807,036 hospitalised cases in England as of October 2021, and these numbers are still increasing especially during winter (AMS, 2021; NHS, 2021).

According to Guest et al (2020), There are six main categories that account for 80% of all HAIs. Respiratory tract infection is the highest rate at 22.8%, followed by urinary tract infection at 17.2%, surgical site infection at 15.7%, clinical sepsis at 10/5%, gastrointestinal infection at 8.8%, and bloodstream infection at 7.3%. The leading cause for these infections is bacterial pathogens, including *Escherichia coli* (*E. Coli*), *Staphylococcus aureus* (*S. aureus*), and *Clostridium difficile* (*C. difficile*). The multi-drug resistant forms of some organisms like Methicillin-resistant *Staphylococcus aureus* (MRSA) compound the problem (Creamer et al., 2014).

1.2 Role of the Environment on HAIs

Since the total elimination of microorganisms is practically impossible, research focuses on controlling the environment and managing human activities to mitigate the factors that increase the risk of infection.

According to Loveday et al (2014), the environment can be responsible for up to 20% of all HAIs. It is sensible to infer that controlling the environment and maintaining hygienic conditions (like periodic disinfection, general hygiene, and ventilation) can have a significant impact on pathogen transmission and can thus lead to reducing the infection risk (Morawska et al., 2020a). A Hierarchy of Controls approach can be used to evaluate risk and illustrates the range of approaches, including using engineering to control the environment, that are important in managing exposure to pathogens (Figure 1:1).

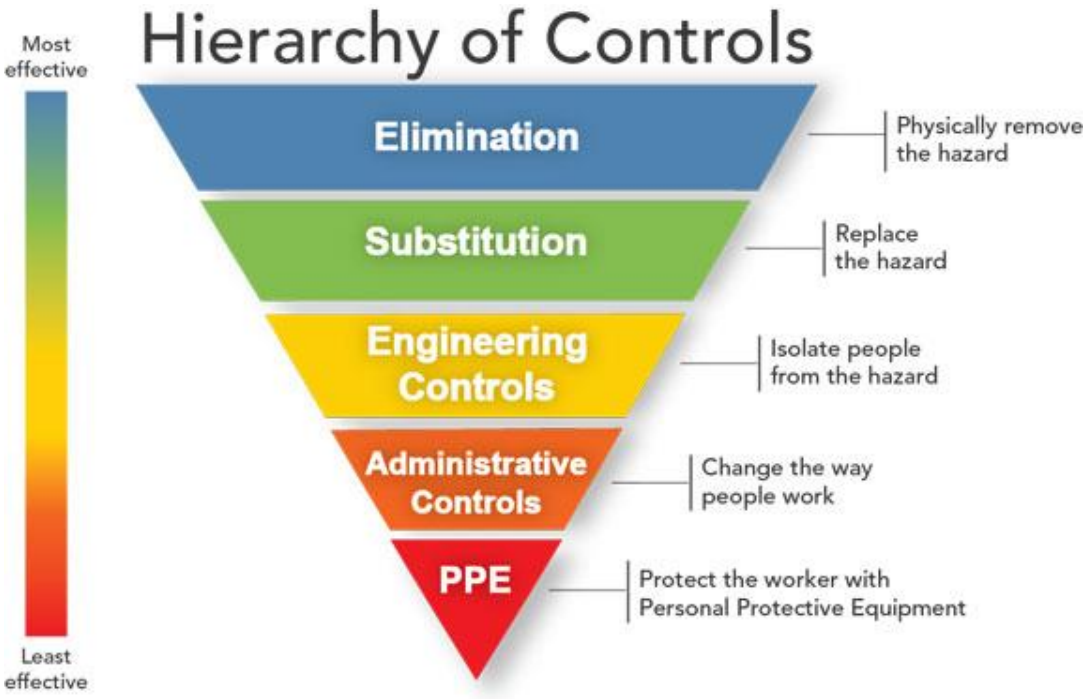

Figure 1:1 Hierarchy of controls (NIOSH-CDC).

The hospital environment is subject to workplace design and layout, operation and maintenance, and hosts multiple interactions between the environment and people. Studies investigating microbial contamination of

the environment have suggested that a wide range of factors may influence the presence of microorganisms, including indoor air quality (IAQ) parameters such as temperature, relative humidity and ventilation; staff activities, patient status and visitor numbers; and surface types, including how and when they are cleaned (CDC, 2003; Dancer et al., 2008; Park et al., 2013; Hathway et al., 2013; Méheust et al., 2013; Hang et al., 2014; Yang et al., 2014; Sajjadi et al., 2016; Demirel et al., 2017). These factors play a significant role in the transmission of several microorganisms, including *Staphylococcus spp*, which could cause HAIs (La Fauci et al., 2017). Hospitalized patients are at a raised risk of being exposed to airborne pathogens, especially in respiratory wards (Chughtai et al., 2019). As well as posing an inhalation risk, bioaerosols can survive and then deposit on the inanimate surfaces at some time and distance from being released by the source (Wilson, et al., 2021).

Although there is a greater focus on transmission risk via hands and surfaces (fomites) in most healthcare settings, airborne microorganisms in hospitals have been associated with several HAIs and various measures of indoor air quality (IAQ) parameters such as (temperature, relative humidity, Carbon dioxide (CO_2), particle mass concentration and particle size) have been linked to pathogen survival or mitigation of pathogen spread.

Studies have also shown that microorganisms in the air can deposit onto surfaces and may be a route to fomite transmission. A qualitative link between microorganisms in the air and on surfaces has been shown in a number of studies in terms of a percentage of positive sampling (Sexton et al., 2006; Carvalho et al., 2007; Best et al., 2010; Creamer et al., 2014; Shimose et al., 2016; Kim et al., 2016a; Killingley et al., 2016). There is a tenuous quantitative correlation for microorganism load in the air and on surfaces shown by several studies (Alberti et al., 2001; Brunetti et al., 2006; Huang et al., 2013; Bonnal et al., 2015; Gheith et al., 2015). Previous work performed active and passive air sampling and also surface sampling at the same time in an intensive care unit (ICU) (Smith et al., 2018a). The study showed that there was a closer relationship between passive air sampling and surface bioburden than there was between active sampling and surface bioburden, and hence passive air sampling was more likely to be useful as a

proxy for infection risk. The relationship between microorganisms in the air and on surfaces in hospital environments remains uncertain and more quantitative data in different settings and varying conditions is required to assess the correlation between air and surfaces contamination and to better understand the factors that affect the spread of airborne pathogens (King et al., 2015; Chia et al., 2020).

Studying the quantitative relationships between the concentration of airborne microorganisms and the surface bioburden in the hospital environment and understanding the manner in which they spread throughout the environment is imperative. Also, understanding the effect of the hospital environment on this relationship can not only lead to understanding this phenomenon but also to reducing the risk of infection through simple procedures to manage exposure to pathogens. The focus of this research is on characterising the factors that influence the concentration of microorganisms in air and their deposition on surfaces over time in hospital environments.

1.3 Transmission of infectious diseases

Microorganisms in the air can originate from the respiratory system due to people coughing, breathing or talking (Asadi et al., 2019). Other sources include aerosolisation from diarrhoea and vomiting (Hathway et al., 2013), from water and drainage systems (Montagna et al., 2017), and through certain clinical procedures (Dancer, 2014). They can also originate through resuspension of microorganisms that may be carried on skin squame through healthcare worker (HCW) activities such as bedmaking or moving curtains (Hathway et al., 2013) and from outdoor environments (Ziaee et al., 2018). Where these microorganisms are pathogens, they can cause HAIs that can be transmitted directly person-to-person, through the air, due to contact with surfaces and fomites in the environment. Figure 1:2 shows the relationship between air and surface routes that can lead to infections in patients, especially those who are immunocompromised.

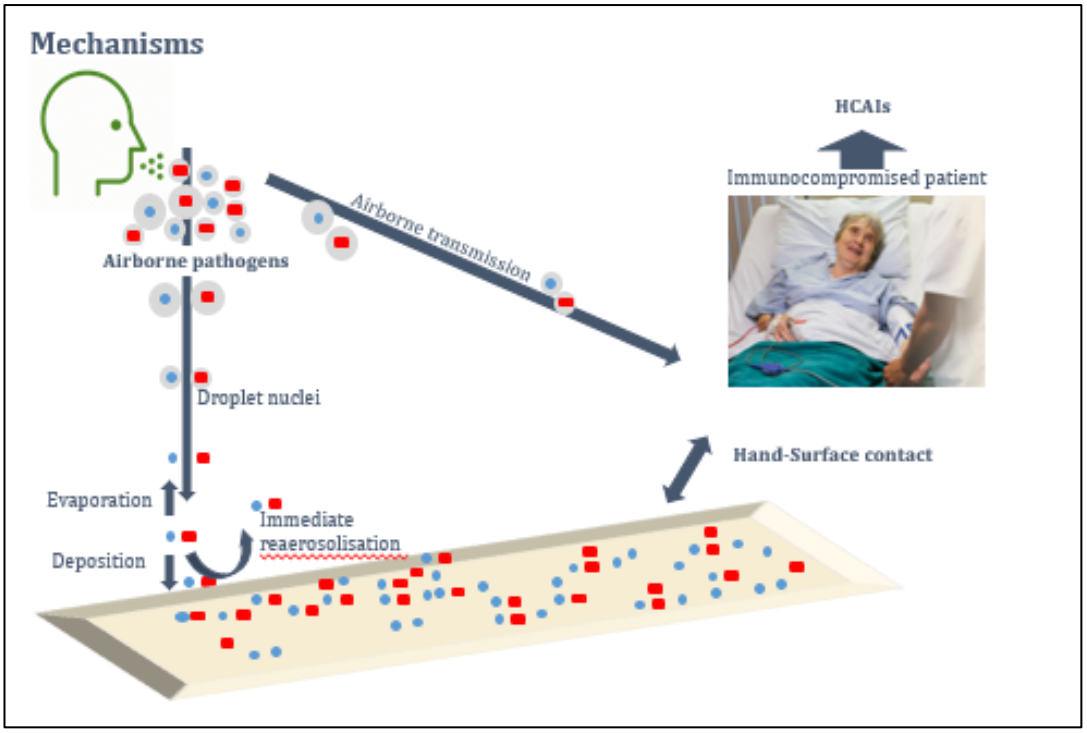

Figure 1:2 Air-surface transmission routes of infectious respiratory diseases

1.3.1 Aerosols and droplets route

Microorganisms from a respiratory source can be transmitted as a ballistic droplet that travels from a patient to an immunocompromised person's mouth, eyes or nostrils. They can also be transmitted in the form of exhaled respiratory aerosols lingering in the air that can be inhaled by an immunocompromised person even after being suspended in the air for hours (Miller et al., 2020).

Respiratory droplets originating from saliva and respiratory secretions can be transmitted through coughing, talking, or even just breathing (Tang et al., 2011). These droplets have a diameter from less than 1µm to over 100µm, where the smaller ones rapidly desiccate to between 40% and 20% of their original diameter, resulting in a residue called a droplet nuclei. Some of these droplet nuclei can be suspended in the air for long periods of time and are thus considered airborne. Several clinicians believe that droplet nuclei and aerosols are the same. The terms and definitions of some of the droplet, droplet nuclei, aerosols, and particles have been contested by researchers with different backgrounds (Tang et al., 2021). Table 1:1 illustrates the

relevant terms and their definitions according to different conceptions among clinicians.

Table 1:1 Differences between clinicians, aerosol scientists and the public in the understanding of airborne terminology (Tang et al., 2021).

Term	Clinicians	Aerosol scientists	General public
Airborne	Long-distance transmission, such as measles; requires an N95/FFP2/FFP3 respirator (or equivalent) for infection control	Anything in the air Collection	Anything in the air
Aerosol	Particle <5 mm that mediates airborne transmission; produced during aerosol- generating procedures and also requires an N95 respirator	Collection of solid or liquid particles of any size suspended in a gas	Hair spray and other personal/cleaning products
Droplet	Particle >5 mm that falls rapidly to the ground within a distance of 1-2 m from source; requires a surgical mask for infection control	Liquid particle	What comes out of an eyedropper
Droplet nuclei	Residue of a droplet that has evaporated to <5 mm; synonymous with 'aerosol'	A related term, 'cloud condensation nuclei', refers to small particles on to which water condenses to form cloud droplets	Never heard of!
Particle	Virion	Tiny solid or liquid 'blob' in the air	Like soot or ash

One of the essential issues to consider is the size of the exhaled particles. Particulate matter of size < 5 µm falls within the range of bioaerosols that may be suspended in the air for over 15 minutes (Mingotti et al., 2020). However, the ability to stay suspended in the air does not depend only on the size but also the surrounding environment, the momentum at which it was expelled, the speed and turbulence of the air, the temperature of the room, and the humidity. The air flow parameters can be a deciding factor leading to several particles that are over 5 µm in diameter being transmitted to distances much greater than the previously conceived 1-2 metres within which particles were believed to deposit on the ground and surfaces. A large number of respiratory infectious diseases are considered to be transmitted between individuals via both airborne routes and by large droplets (Xie et al., 2007). This indicates that it is necessary to consider both "droplets" and "aerosols" to be within the range of sizes of interest. The environmental

factors and airflow parameters must be considered in concluding the target sizes. Thus, the term “droplets” is used for particles that can deposit or fall on surfaces due to the gravitation force or the momentum at which they were expelled. Aerosols, on the other hand, are particles that do not fall but stay suspended in the air for over 30 minutes due to various reasons, including size and environmental factors (Tang et al., 2021). The term particles will be the general term referring to both droplets and aerosols. Figure 1:3 shows a range of respiratory particles that may spread over a distance.

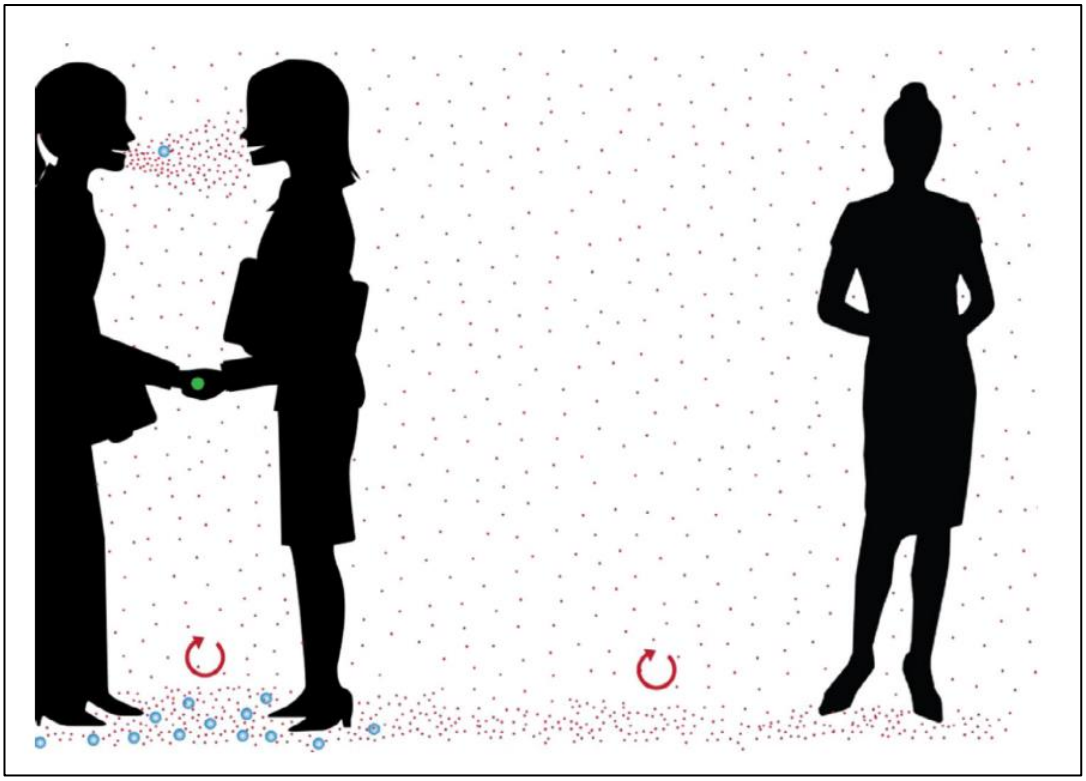

Figure 1:3 Spread of droplets (blue particles) and aerosols (red particles) (Tang et al., 2021).

There have been several studies that have looked at the role of air in the transmission of infections, but there is little quantitative analysis of how deposition from the air onto surfaces can pose a risk, so research investigating microorganisms in healthcare environments would be significantly beneficial (King et al., 2016). The research needs empirical evidence to clarify the effect of environmental conditions and particle size of the droplet on the suspension period and the travelling distance.

Microorganism concentration in the air can be quantified either by active or passive sampling methodology (Wong et al., 2011; Shimose et al., 2016; Smith et al., 2018a). Active sampling draws the air into a liquid or agar plate, while passive sampling collects the airborne microorganism onto open Petri dishes by gravity sedimentation for a period of time. This is discussed in detail in chapter 2 (2.3.2).

1.3.2 Contact route

The contact transmission route contains all infectious diseases where the susceptible patient is in direct or indirect contact with the source of the pathogen. Direct contact transmission from donor to recipient occurs mainly via contaminated hands (Pittet et al., 2006; Van-Kleef et al., 2013). Inanimate surfaces work as reservoirs and are the middle process of indirect contact transmission where an immunocompromised person may be in contact with a contaminated surface and then touch their mouth, eyes, or nostrils. This route can also happen when the patient or healthcare worker has contaminated hands and touches a wound site. In both direct and indirect contact transmission, hand hygiene plays an essential role in reducing the spread of infection (Hathway et al., 2011). Cleaning is also essential to keep the surface bioburden within the accepted level that is suggested to be <5 cfu/cm^2 for general wards and <2.5 cfu/cm^2 for critical care units (White et al., 2008; Bogusz et al., 2013). Surface sampling is typically carried out using swabs, sponges or contact plate/dipslides (Jomha et al., 2014; Picot-Guéraud et al., 2015). This is discussed in detail in chapter 4 (4.1.3)

1.4 Healthcare Building Environment

The healthcare building environment refers to the environmental factors that can affect the transmission of pathogens. It can be classified into two main types: human factors and physical factors.

1.4.1 Human factors

Human factors like the staff activities, visitors presence, patient status, and cleaning policies can influence the airborne microbial load inside a building (Talon et al., 2008; Hathway et al., 2013; La Fauci et al., 2017). Patients and staff may generate or transmit bacteria and fungi due to their presence, movement, shedding, or activities of a HCW. Studies in the literature present measurements with high variability in results considering studies conducted in different locations, while only a few studies show fluctuation in measurements with time at the same location (Hathway et al., 2013). This demonstrates the complexity of the interactions with microorganisms, particles and CO_2 concentrations affected by the number of people and the activities taking place. However, while the interaction between the presence of humans and their activities with microorganisms, particles and CO_2 concentrations is apparent, the relationship of the deposition rate of microorganisms with these factors is uncertain.

1.4.2 Physical factors

Building environment factors include ventilation type and regime, structures design and layout, location, and IAQ parameters such as temperature, relative humidity, CO_2 level (which reflects the ventilation rate and occupancy), particle mass concentration and particle size. These factors are essential for the health and wellbeing of those in hospitals and may also influence the bioburden in the environment by affecting the survival, deposition onto surfaces and transmission of pathogens (Møretrø et al., 2010; Dougall et al., 2019). Airborne microorganisms in hospitals have been associated with several HAIs, and various measures of IAQ have been linked to pathogen survival or mitigation of pathogen spread.

Ambient air temperature and relative humidity (RH) are usually measured in indoor environments to understand the thermal comfort and well-being of occupants. However, both parameters are also linked with the survival of microorganisms, with humidity a particular concern. Many bacteria and fungi favour more humid conditions (Møretrø et al., 2010; Zoz et al., 2016). However, there is evidence that virus survival increases at humidity below 40% RH (Marr et al., 2019). Guidance varies around the world, but

temperatures within 18-28 °C and humidity in the range of 40-60% RH are commonly recommended. (Department of Health, 2007)

CO_2 is related to the exhaled breath of occupants and is frequently measured in indoor environments as an indicator of ventilation rates and occupancy. A number of studies have also shown that ventilation rates expressed through CO_2 concentrations can be used to evaluate airborne infection risk (Rudnick and Milton, 2003; Vouriot et al., 2021).

Airborne particles provide a general measure of indoor air quality and can be related to indoor sources and activity or outdoor conditions (Licina et al., 2016). Some studies suggest using airborne particles as a proxy for the cleanliness of the air, including to commissioning of specialised hospital ventilation systems (Department of Health / Estates and Facilities Division, 2007).

Although the correlation between IAQ parameters and microorganism prevalence and survival has been studied for decades, there are conflicting results (Augustowska and Dutkiewicz, 2006; Hsu et al., 2012; Hathway et al., 2013; Mirhoseini et al., 2015; Huang et al., 2017; Osman et al., 2018; Božić et al., 2019) and it is not clear which parameters may be significant and how they interact together. If there are significant and consistent relationships between the microbial load in the air and IAQ parameters, this could allow IAQ to be used as a proxy for evaluating the likelihood of microorganisms being present in the air. Thus, understating and quantifying the effect of these factors on airborne microorganisms can enable healthcare professionals to control these factors and mitigate the infection risk.

1.5 Modelling aerosol infection risk

Modelling transmission risk is beneficial for assessing the factors that influence exposure to microorganisms and understanding how the environment can be designed and managed to minimise infection risks. Wells and Riley introduced an airborne pathogens transmission model based on Poisson probability distribution (Wells et al.1955). This model presents a “quantum of infection” representing the level of virulence of

pathogens that leads to human infection but not all inhaled infectious particles will result in infection. This model was then amended by Gammatoni and Nucci (Gammaitoni and Nucci, 1997), who was able to combine the Wells-Riley model with the room ventilation, deposition, and decay rates taking into consideration the influence of a non-steady-state quanta generation on the risk of Tuberculosis (TB) in an indoor environment. The improved model has been used in numerous studies including for predicting the influence of ventilation on risks of airborne infection in a hospital ward over time (Noakes and Andrew Sleigh, 2009) and assessing outbreaks including the SARS-CoV-2 infection risk based on the quanta generation rate in a choir outbreak (Miller et al., 2021).

Using modelling techniques is one of the best approaches to understand and mitigate the infection risk. There are several Computational Fluid Dynamics (CFD) studies that have modelled deposition (Hathway et al., 2011; King et al., 2017a; Wilson, M.-F. King, et al., 2021) and only a small number of quantitative microbial risk assessment (QMRA) studies have modelled surface contact risks as well as airborne risks (Lopez et al., 2013; WHO, 2016; Xie et al., 2017). However, most of these studies either do not consider the deposition rate and the factors that affect the relationship between microorganisms in the air and on surfaces in hospital environments or assume a constant deposition rate. There are only a small number of CFD studies (King et al., 2017a; Wilson, M.-F. King, et al., 2021) that include the particle deposition and influence of the airflow explicitly in models. The loss rate of airborne pathogens due to deposition onto surfaces with consideration to human factors and physical factors like indoor air quality parameters is one of the factors that can be controlled in order to mitigate the infection risk, and it is still not well-understood in the literature.

The COVID-19 pandemic has raised awareness of the importance of the built environment in the transmission of infection. For many diseases, it is difficult to determine the relative importance of airborne and surface transmission, but the two are connected; airborne microorganisms deposit onto surfaces. Hospital environment parameters such as ventilation have been shown to impact airborne transmission and there is a small amount of evidence that it could also reduce risks of transmission via surfaces.

However, there is a lack of quantitative data on factors affecting deposition of microorganisms from the air and the impact of these factors to mitigate the surface transmission risks. This data is needed to be able to model the effect of interventions such as changes to ventilation and cleaning regiemes. The research in this thesis can provide some of this missing data on the impact of environmental parameters on the deposition rate of airborne microorganisms onto surfaces. It also provides new modelling and experimental tools to develop the methodologies used for assessing the concentration of deposited bioaerosols on surfaces.

1.6 Aims and Objectives

The aim of this research is to characterise the spatial and transient relationships between microorganisms (predominately aerobic bacteria) in air and on surfaces and to understand the factors that influence this relationship in both controlled chamber and hospital environments.

1.6.1 Research Questions

This research addresses the following research questions:

- What are the physical and human factors that influence microorganisms in the air and on surfaces?
- What is the most appropriate way to measure microorganisms in air and on surfaces and how can the transient effects be captured?
- How does deposition of microorganisms onto surfaces vary in different healthcare settings and compared to controlled chamber experiments?
- How do bioaerosols contribute to the bioburden on surfaces in hospitals through an environmental route?
- Can simple mathematical relationships be developed to quantify the influence of airborne microorganisms on surface contamination?

1.6.2 Objectives

The main objectives are:

1- To evaluate the prevalence of airborne microorganisms in hospital environments and approaches by which this is measured.
2- To characterise the relationships between indoor air quality parameters and airborne microorganisms in hospital environments.
3- To externally validate existing equations and develop a model that can be used to quantify the relationship between microorganisms in the air and on surfaces.
4- To characterise the transient bioburden in the air and on surfaces in hospitals.
5- To develop experimental tools that enable time series surface sampling without human intervention to quantify the deposition rate of airborne microorganisms over time.
6- To quantify the effect of the environment and the ventilation rate on the spatiotemporal deposition rate of microorganisms on surfaces.
7- To assess how the quantitative relationship between airborne microbial load and surface bioburden can be used in practice with consideration of HCWs activity and environmental conditions.

1.7 Thesis Scope and Structure

The work in this research is aligned to the EPSRC funded Hospital Environment Control, Optimisation and Infection Risk Assessment (HECOIRA) project. The HECOIRA project aims to develop novel computational-based tools to assess, monitor and control patient environments in hospitals for infection control, comfort and wellbeing. One of the main targets of this research is to provide some of the microbial data required to validate models and define input parameters to use in infection risk models. The rest of the thesis is organised as follows:

Chapter 2 (The prevalence of airborne microorganisms in hospital environments): This chapter presents a systematic review that collects data from research articles that study airborne microorganisms in hospital environments. It shows the different sampling methodologies used and the variety of approaches that are followed to collect and present results. This data provides guidelines for future research to obtain quantitative results in a

more unified manner taking into consideration human and environmental factors.

Chapter 3 (The relationship between indoor air quality parameters and airborne microorganisms in hospital environments.): This chapter shows the correlation between IAQ parameters and airborne microorganisms and identifies the potentially significant parameters using a meta-analysis approach. It discusses the potential impact of controlling IAQ parameters on reducing airborne bioburden, which might in turn, reduce the infection risk from airborne microorganisms. Moreover, it explains the way by which they interact with each other and with other variables with respect to confounding using the directed acyclic graph (DAG) approach.

Chapter 4 (The relationship between airborne microorganisms and surface bioburden in hospital environments): This chapter summarises the air and surface sampling techniques that are used in hospital studies. It considers published data that has measured both air and surface bioburden and provides a new general linear predictive model of airborne bioaerosols level based on the surface bioburden considering guidelines for cleanliness.

Chapter 5 (Measuring airborne microorganisms and surface bioburden in a hospital environment): This chapter builds on the lack of timeseries data identified in chapters 2-4 and studies the transient relationships between airborne and surface bioburden, considering the presence and activity of healthcare workers. It measures the fluctuations in microbial load over time in air and on surfaces and provides recommendations for the accepted level of airborne microorganisms in hospital wards. This process is based on the deposition rate of airborne micrograms on surfaces and the cleanliness threshold.

Chapter 6 (Introducing Multiplate air passive sampler to measure deposition rate of airborne microorganisms overtime): This chapter develops and validates a novel configurable device that can expose a plate to air for a pre-determined interval, cover it, and autonomously expose a different one to gain an automated method of sampling at preconfigured intervals without human intervention. This new tool can provide automated timeseries surface samples to give reliable and accurate data while

investigating the influence of different ventilation rates on spatial bioaerosols.

Chapter 7 (Deposition rate of microorganisms under the steady-state condition in a controlled microbiological chamber): This chapter quantifies the loss rate due to deposition onto surfaces over time with considerations for varied ventilation and different locations. It provides more data to understand more the effect of ventilation rate on deposition rate.

Chapter 8 (Conclusions and Further Work): This chapter presents the final conclusions and recommendations for potential future development in the area of microorganism transmission in healthcare environments.

2 The prevalence of airborne microorganisms in hospital environments

2.1 Introduction

The hospital environment plays a crucial role in managing HAISs by influencing the survival and spread of pathogens (Perdelli et al., 2008). The hospital environment includes the workplace design and layout, IAQ parameters such as (temperature, relative humidity, CO_2, particle mass concentration and particle size), type of ventilation (natural or mechanical), how the ventilation is designed and its flow rate, activities of people (staff, patients and visitors), surfaces type etc. Thus, we need to ascertain the current knowledge around the effect of hospital environmental parameters on the prevalence and species of airborne microorganisms. Carrying out a systematic review is an excellent approach to achieve this goal. This will enable the following questions to be addressed: (i) is there any relationship between hospital environment factors and airborne microorganisms; (ii) how do they interact with each other; (iii) is there sufficient quantitative data to perform an effective meta-analysis and to understand the relationship between them?

2.2 Methodology

2.2.1 The systematic review

Throughout this chapter, the term “review” refers to a systematic review, and the term “microorganisms” refers to bacteria, fungi, and viruses. The review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance (Liberati et al., 2009; Moher et al., 2009). For the identification phase, three electronic databases (Web of Science and Scopus and PubMed) were searched systematically from inception to October 2020 using keywords “air, sampling, hospital, environment, AND contamination”. Full-text articles published in English that include air sampling data for microorganisms in patient’s areas of hospitals were selected for inclusion. The studies that carry out air sampling in hospital rooms with specialist ventilation ≥ 10 air changes per hour (ACH) or

areas undergoing construction or renovation were excluded. The previous work finds that construction and renovation can affect the concentration of airborne microorganisms and cause it to fluctuate, and this is unlikely to be representative of normal conditions in a hospital (Wirmann et al., 2018). In operating theatres, isolation rooms or other specialist areas the high rate of ventilation reduces the airborne concentration, which makes it difficult to understand the effect of other factors (Nielsen, 2009). High ventilation rates of 20-25 ACH is used in the operation room, while in wards, a rate of 6ACH or less is more common (Department of Health, 2007).
This resulted in the identification of (114) articles that were included for qualitative synthesis to gather data on IAQ parameters, infection risk, temporal factor, sources of infection, spatial factor and other factors (Figure 2:1).

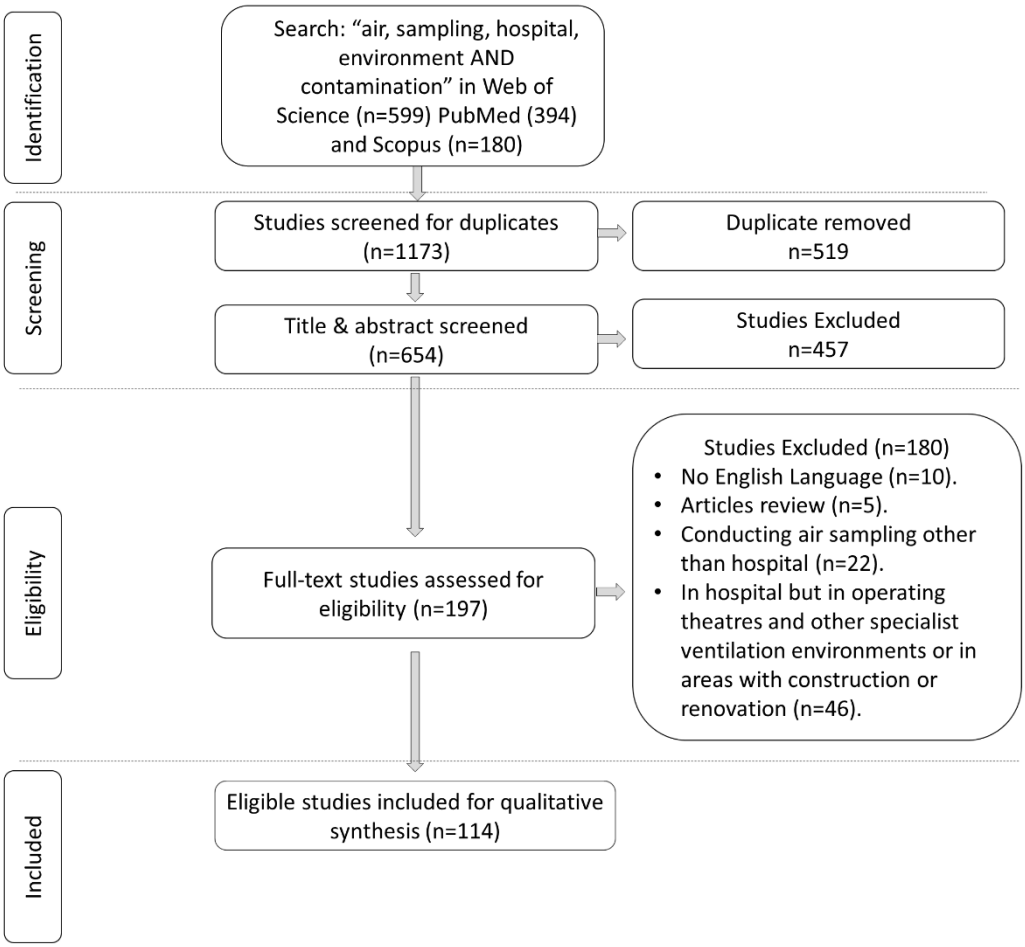

Figure 2:1 Flow chart of the systematic review, search strategy and exclusion and eligibility criteria.

2.3 Results and discussion

2.3.1 Overview of included studies

This review identified (114) studies that performed air sampling and that meet the inclusion criteria. The hospital environment has several parameters that can be categorised into six groups (IAQ parameters, infection risk, temporal factor, sources of infection, spatial factor and other factors) (Table **2**:**1**).

Before going through these categories, it is useful to discuss the type of air sampling, targeted microorganisms and their identification as described in the reviewed paper. Studies that perform air sampling are distributed across five decades. Figure 2:2 shows that the subject is gaining an increased attention every decade and that the most recent ten years include the highest number of studies that are interested in airborne microorganisms in hospital settings. Note that this search was carried out before the COVID-19 pandemic and does not include any of the COVID-19 literature.

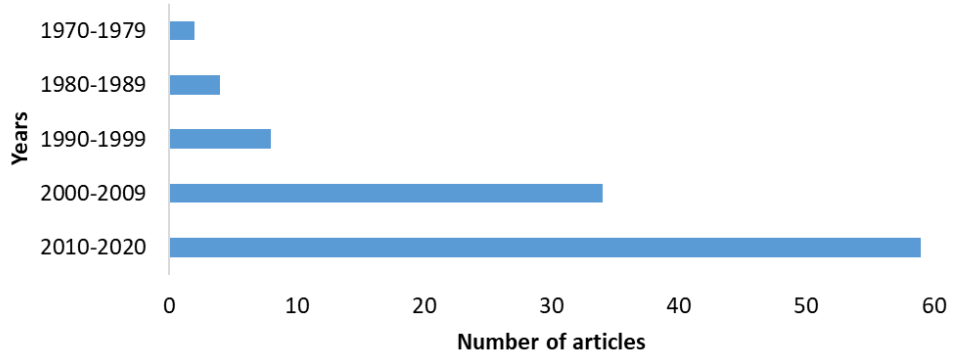

Figure 2:2 Studies of airborne microorganisms over five decades.

Table 2:1 The relationship between airborne microorganism levels and hospital environmental factors.

Investigating the relationship between airborne microbial concentration and or in:		Presenting data as		References
		Quantitative	Semi-quantitative	
Indoor air quality parameters	Temperature	7	1	(Augustowska and Dutkiewicz, 2006; Park et al., 2013; Azimi et al., 2013; Yang et al., 2014; Mirhoseini et al., 2015; Picot-Guéraud et al., 2015; Sajjadi et al., 2016; Osman et al., 2018).(Augustowska and Dutkiewicz, 2006; Viegas et al., 2011; Park et al., 2013; Cavallo et al., 2013; Azimi et al., 2013; Matoušková and Holy, 2014; Yang et al., 2014; Picot-Guéraud et al., 2015; Mirhoseini et al., 2015; Sajjadi et al., 2016)
	Relative humidity	9		(Augustowska and Dutkiewicz, 2006; Park et al., 2013; Azimi et al., 2013; Yang et al., 2014; Mirhoseini et al., 2015; Sajjadi et al., 2016; Demirel et al., 2017; Osman et al., 2018; Božić et al., 2019).
	Carbon dioxide	3	0	(Hsu et al., 2012; Park et al., 2013; Yang et al., 2014)
	Particle mass concentration	3	0	(Hsu et al., 2012; Yang et al., 2014; Huang et al., 2017)
	Particulate matter of size	3	0	(Hathway et al., 2013; Mirhoseini et al., 2015; Huang et al., 2017)
	Rain & wind	2	2	(Cavallo et al., 2013; Yang et al., 2014; Niaré-Doumbo et al., 2014; Martínez-herrera et al., 2016)
Infection risk	Occurrence of infection (e.g. respiratory, burn, neonatal sepsis)	6	18	(Grieble et al., 1970; Stone and Das, 1986; Sarfati et al., 1994; Bartlett et al., 1997; Moore et al., 2002; Panagea et al., 2005; Booth et al., 2005; Kronman et al., 2007; Perdelli et al., 2008; Gehanno et al., 2009; Best et al., 2010; Montagna et al., 2012; Lee et al., 2012; Shrestha et al., 2012; Munoz-Price et al., 2013; Barbut et al., 2013; Ao et al., 2014; Arcy et al., 2014; Jomha et al., 2014; Gheith et al., 2015; Bischoff et al., 2016; Shimose et al., 2016; Kim et al., 2016a; La Fauci et al., 2017)
	Surface bioburden	3	10	(Alberti et al., 2001; Brunetti et al., 2006; Sexton et al., 2006; Carvalho et al., 2007; Best et al., 2010; Huang et al., 2013; Creamer et al., 2014; Gheith et al., 2015; Bonnal et al., 2015; Shimose et al., 2016; Kim et al., 2016a; Killingley et al., 2016; Smith et al., 2018a)

	Investigating the relationship between airborne microbial concentration and or in:	Presenting data as		References
		Quantitative	Semi-quantitative	
Sources of microbes	Patient's status	3	10	(Quinn et al., 1984; Arnow et al., 1991; Döring et al., 1993; Alberti et al., 2001; Sexton et al., 2006; Carvalho et al., 2007; Talon et al., 2008; Gurgui et al., 2011; Munoz-Price et al., 2013; Cheng et al., 2015; Bonnal et al., 2015; Kim et al., 2016a; Shimose et al., 2016)
	Human activities (nursing care, patients, housekeeping)	11	8	(Schmidt et al., 1984; Arnow et al., 1991; Ensor et al., 1996; Rainer et al., 2001; Shiomori et al., 2002; Moore et al., 2002; Khojasteh et al., 2007; Talon et al., 2008; Andersen et al., 2009; Liguori et al., 2010; Wong et al., 2011; Best et al., 2012; Lee et al., 2012; Tekİn et al., 2013; Hathway et al., 2013; Hsueh et al., 2014; Verani et al., 2014; Cheng et al., 2015; Killingley et al., 2016)
	Outdoor airborne microbes	2	2	(Tambekar et al., 2007; Nandalal and Somashekar, 2007; Ao et al., 2014; Rostami et al., 2017)
	Waste disposal sites	1	0	(Verani et al., 2014)
Spatial factors	Hospital and other indoor buildings (e.g. school, library, office, farm, etc.)	3	2	(Nunes et al., 2005; Zorman and Jeršek, 2008; Sabino et al., 2014; Memon et al., 2016; Demirel et al., 2017)
	Different hospitals in the same country	3	1	(Panagopoulou et al., 2002; Farmaki et al., 2007; Faires et al., 2012; Martínez-herrera et al., 2016)
	Different units in the same hospital	12	7	(Chakrabarti et al., 1992; Jaffal et al., 1997; Panagopoulou et al., 2002; Al-Shahwani, 2005; Perdelli et al., 2006; Brunetti et al., 2006; Nandalal and Somashekar, 2007; Falvey and Streifel, 2007; Ríos-Yuil et al., 2012; Tekİn et al., 2013; Gaudart et al., 2013; Me´heust et al., 2013; Azimi et al., 2013; Mirhoseini et al., 2015; Saadoun et al., 2015; Fekadu and Getachewu, 2015; Martínez-herrera et al., 2016; Gizaw et al., 2016; Memon et al., 2016)

	Investigating the relationship between airborne microbial concentration and or in:	Presenting data as		References
		Quantitative	Semi-quantitative	
	Different locations in the same ward or room	7	9	(Rainer et al., 2001; Khojasteh et al., 2007; Krishna et al., 2007; Lee et al., 2007; Talon et al., 2008; Barbolla et al., 2008; Gehanno et al., 2009; Faires et al., 2012; Muzslay et al., 2013a; Gaudart et al., 2013; Méheust et al., 2013b; Matoušková and Holy, 2014; Bonnal et al., 2015; Mirhoseini et al., 2015; Killingley et al., 2016; Kim et al., 2016a)
	the layout of ward or room	0	1	(Moore et al., 2010)
Temporal factors	Seasonal influence	7	8	(Grieble et al., 1970; Hospenthal et al., 1998; Panagopoulou et al., 2002; Martins-Diniz et al., 2005; Brunetti et al., 2006; Augustowska and Dutkiewicz, 2006; Lee et al., 2007; Farmaki et al., 2007; Falvey and Streifel, 2007; Cordeiro et al., 2010; Cavallo et al., 2013; Park et al., 2013; Ao et al., 2014; Niaré-Doumbo et al., 2014; Gheith et al., 2015)
Temporal factors	Sampling daytime	8	3	(Döring et al., 1993; Rainer et al., 2001; Al-Shahwani, 2005; Augustowska and Dutkiewicz, 2006; Roberts et al., 2008; Ríos-Yuil et al., 2012; Creamer et al., 2014; Fekadu and Getachewu, 2015; Killingley et al., 2016; Martínez-herrera et al., 2016; Gizaw et al., 2016)
Other factors	Other indoor airborne microbes	8	0	(Jaffal et al., 1997; Carducci et al., 2011; Hathway et al., 2013; Park et al., 2013; Verani et al., 2014; Fekadu and Getachewu, 2015; Sajjadi et al., 2016; Demirel et al., 2017)
Other factors	Different sampler	3	0	(Nunes et al., 2005; Rodrigues and Araujo, 2007; Me´heust et al., 2013)
Other factors	Type of ventilation (LAF) and HEPA filtration	7	7	(Bodey and Johnston, 1971; Barnes and Rogers, 1989; Alberti et al., 2001; McLarnon et al., 2006; Crimi et al., 2006; Lee et al., 2007; Rodrigues and Araujo, 2007; Falvey and Streifel, 2007; Crimi et al., 2009; Lee et al., 2012; Shimono et al., 2012; Brun et al., 2013; Picot-Guéraud et al., 2015; Bonnal et al., 2015)
	Total			114

2.3.2 Air sampling approaches

Airborne microorganisms can be detected using active sampling, passive sampling or both approaches. Each approach has its own advantages and disadvantages. The review shows that 76% of the studies used an active sampling method compared to 11% using passive sampling. Only 13% used both methods, meaning that there is little empirical data available from real settings to compare between the two approaches. These results might be due to the fact that recommendations for air control are based primarily on the volumetric measurements in cfu/m^3 (Pasquarella et al., 2000). This might be the reason that researchers commonly use active sampling instead of passive methods that require an equation to represent the deposition rate of microorganisms because this needs to be converted to cfu/m^3. This is particularly the case for environments with specialist ventilation, such as operating rooms, where active air sampling is often carried out as part of the validation of the ventilation (Napoli et al., 2012).

2.3.2.1 Active sampling

Active sampling devices can be classified into three types depending on their collection mechanism: impingers and cyclones, which both sample into a liquid through different flow methods; Impactors that sample directly onto an agar plate or strip; and filters, which sample onto a permeable membrane (Figure 2:3). The first two types are commonly used for sampling bioaerosols in hospitals. The bio-efficiency of these devices needs to be identified before their usage, so it is recommended to test the selected device with the target pathogen in a lab environment under similar conditions to that of the hospital (Haig et al., 2016).

Collection into liquid can be beneficial where there is a need to sample for long periods or where microbial analysis uses more than one method, while impactors that collect onto an agar plate are easier to use and can size fractionate microbial samples (Haig et al., 2016). The pros of active sampling methods are: (i) being fast ; (ii) sample is not dependent on the local room airflow pattern ; (iii) while sampling results depend on the type of sampler, there are usually negligible differences between results from comparable

instruments from different manufacturers (Nunes et al., 2005, Méheust et al., 2014). However, a study indicates some drawbacks, including: (i) samplers are expensive, noisy and difficult to sterilise; (ii) the sampler or its flow meter must be calibrated; (iii) the room airflow is disturbed due to sampling; (iv) a number of microorganisms may be inactivated by the sampler itself (Haig et al., 2016).

The Centre for Disease Control and Prevention CDC (2003) and several other organisations offer recommendations and guidelines for active sampling. Further considerations, like sampling at different heights (1m, 1.5m), had no significant difference in concentration in a study looking at the spores of *Cladosporium spp* (Ríos-Yuil et al., 2012). However, this may be specific to this scenario; either the room was well mixed, or the devices were not capable of detecting any differences.

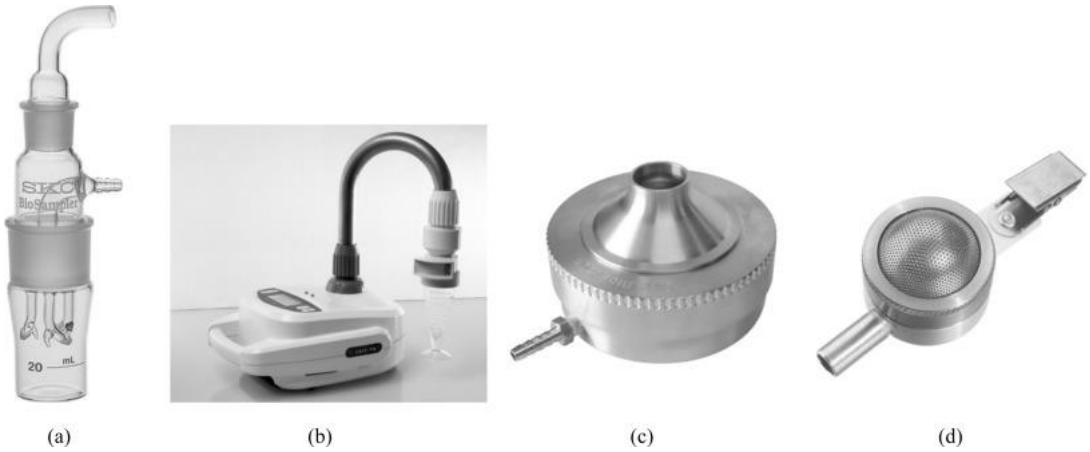

Figure 2:3 (a) SKC BioSampler (impinger); (b) Coriolis sampler (cyclone); (c) SKC BioStage Impactor; (d) SKC Button Sampler (filter) (Haig et al., 2016).

2.3.2.2 Passive sampling

Passive sampling methods depend on gravity sedimentation to collect microorganisms from the air, usually onto an open Petri dish containing a suitable agar (Figure 2:4). Passive sampling is more accessible as it is inexpensive, may be performed in several places at the same time and, contrary to active sampling, is silent so it can be used at night. Pasquarella et al. (2000) provide some guidelines for passive sampling, including a 1/1/1 scheme that suggests sampling for one hour, at one metre height from the

ground and at least one-metre distance from walls and obstacles. Airborne microorganisms that are not removed by ventilation may eventually deposit onto surfaces, and the number that deposit is expected to correlate with the number of microorganisms present in the air. It is hypothesised by a number of studies that there is a relationship between the number of microorganisms measured on surfaces and those measured in the air (Bonnal et al., 2015; Smith et al., 2018a). Understanding this could benefit the evaluation of hospital hygiene; the use of settle plates to assess airborne risk offers an affordable and easy sampling approach.

Using passive sampling alone, however, and presenting the data as cfu/m^3 without a clear mathematical rationale to quantify the relationship between both passive and active samples may not provide reliable results. There are many variables, including ventilation and healthcare activities, that affect the level of airborne microbes and the rate of deposition (Perdelli et al., 2006; Hathway et al., 2013). Deposition rate depends on the size of the microbial particle, with larger particles depositing more readily (Nazaroff, 2016). As shown in experiments with inert particles in domestic environments, there is not a clear relationship between particle size and deposition rate, with factors such as the local airflow patterns affecting deposition (Thatcher, Alvin C.K. Lai, et al., 2002; Miller et al., 2020). It is this uncertainty that has led to the analysis of mathematical relationships between air and surface microorganisms which are detailed in chapters 4, 5, and 6.

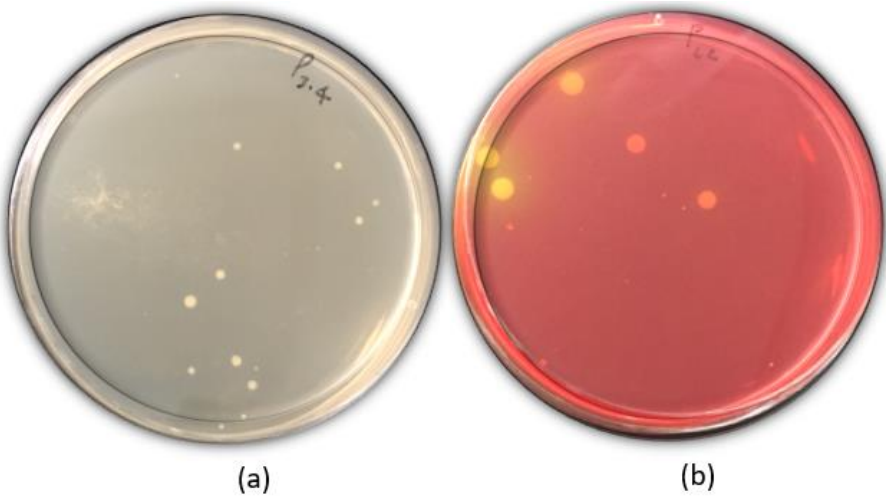

Figure 2:4 Passive sampling (open petri dishes; (a) Tryptone Soya Agar (TSA) and (b) Mannitol salt agar (MSA).

2.3.2.3 Air sampling recommendations

Using the data collected from the literature, these recommendations were observed for effective air sampling in hospital wards that provides reliable unbiased and more generalised data to be used in further analyses and comparisons with existing studies and with hospital environment factors:

- Contamination source concerns: It is known that patients, sinks and bathrooms are sources for microorganisms' generation and it is thus better to place the samplers at least 1m away from these sources to minimise their effect on the collected results, unless these sources are of interest to the researcher's investigations.
- Airflow concerns: To minimise the effect airflow dynamics, samplers should be placed at least 1m away from natural ventilation sources like doors and windows, and mechanical ventilation sources like fans, air conditioning vents, and air extractors. This is to ensure that these factors do not affect the concentration of microorganisms at the time and location of sampling.
- Infection risk concerns: The airborne microorganisms' concentration needs to be studied to assess and to minimise the infection risk. Samplers should be placed at a breathing height that is between 1m and 1.5m depending on whether the patient is generally sitting or lying down, so that the sampler can collect amounts similar to that inhaled by people.
- Errors and fluctuation concerns: To make sure that the sampling results are unbiased, at least two replicates either at the centre of microorganisms' generation sources (patients) or at two diagonally separated locations must be taken for each sample.

2.3.3 Targeted microorganisms

2.3.3.1 Focus of studies

Bacteria are more widely studied (53%) than fungi (42%) or viruses (5%) in hospital settings; *S. aureus* and airborne microbial concentration measured as aerobic colony count (ACC) featured in 27 and 26 articles, respectively. Fungi have also attracted researchers' interest where *Aspergillus fumigatus*, *Aspergillus spp* and total airborne fungi (TF) were reported in 21, 18 and 16 articles, respectively. Viruses were less frequently studied as only three

researchers conducted air sampling to find Torque teno virus as shown in (Table 2:2). One of the virus studies presented a quantitative relationship between the measles virus in the air and on surfaces (Bischoff et al., 2016). It should be noted that this section of the review was conducted prior to COVID-19 and that there were several new studies that sample SARS-CoV-2 virus in air and on surfaces in hospitals that are not included. To reduce the time and cost of screening microorganisms in the hospital environments, it would be significantly beneficial to find the correlation between these types of microorganisms.

Table 2:2 The total number of studies targeting different types of microorganisms

Bacteria	N.	Genera of Fungi	N.	Virus	N.
Staphylococcus aureus	27	*Aspergillus fumigatus*	21	*torque teno virus*	3
ACC	26	*Aspergillus spp*	18	*human adenovirus*	2
Pseudomonas aeruginosa	12	TF	16	*norovirus*	2
Staphylococci (CNS)	7	*Aspergillus flavus*	15	*human rotavirus*	2
Enterobacter	6	*Aspergillus niger*	15		
Acinetobacter baumannii	6	*Penicillium spp.*	11		
Escherichia coli	5	*Aspergillus terreus*	8		
Klebsiella	5	*Cladosporium*	8		
Clostridium difficile	5	*Candida*	6		
Bacillus spp	3	*Rhizopus*	6		
Enterococcus	2	*Fusarium spp*	6		
Proteus	2	*Aspergillus versicolor*	5		
Burkholderia cepacia	2	*Aspergillus nidulans*	4		
Micrococci	2	*Yeast*	4		
GNB	2	*Aspergillus clavatus*	3		
Streptococcus pyogenes	2	*Mucor spp.*	2		
Staphylococcus saprophyticus	2	*Mucorales spp*	2		
Enterobacteriaceae	2	*Chrysosporium sp.*	2		
		Alternata	2		
		Alternaria spp.	2		

N, number of studies; ACC= airborne microbial concentration measured as an aerobic colony count

2.3.3.2 Microorganism identification methods

The results of the review show a significant diversity of microorganisms have been measured in hospital air, and these display a range of environmental persistence as well as different detection criteria. It is crucial to study the viability of a microorganism to determine whether it presents a risk of infection. However, there is no single method that can reliably determine the accurate viability of a microorganism (Me´heust et al., 2013). Two types of methods are recommended to indicate the viable fraction. Culture-based methods provide quantitative and qualitative data but are affected by media type and incubation time. Cytometry methods can provide an assessment of viability by showing a ratio of live cells. According to Rainer et al (2001), using culture-based methods, there is no significant difference between dichloro-glycerol agar and malt extract agar on TF load. However, the mean concentrations of viable TF that are found using cytometry methods were 50% higher than concentrations found using a culture-based method that uses malt extract agar (Me´heust et al., 2013). This recovery efficiency is not generalisable for all microorganisms but highlights that the method of detection should be considered when drawing conclusions from the results.

2.3.4 Sources of Microorganisms

2.3.4.1 Human sources

The status of the patients (not colonised, infected and/or colonised) has a significant effect on the bioaerosol load for several types of microorganisms. *Aspergillus spp* and MRSA were more widely studied than other pathogens, as shown in (Table **2**:**3**).

There is evidence that clinical care activities can influence airborne pathogen loads in a ward by liberating significant quantities of bacteria, raising the importance of investigating the patient's activities in diverse events (Hathway et al., 2013). For example, the number of positive cultures of *Burkholderia cepacia* increased during and after receiving physiotherapy to 45% compared with 16% before physiotherapy, which is usually done for patients with cystic fibrosis (Ensor et al., 1996). Dressing changes and nasal decolonisation with mupirocin were shown to increase the level of positive

air sampling and positive sampling of surfaces by around 18% and 12%, respectively (Talon et al., 2008).

Table 2:3 Summary of studies that consider the effect of patients' infection status on the level of the airborne microorganisms.

Reference	Type of microbes or disease	Patient status	P-value	Investigating HAISs
(Quinn et al., 1984)	*Corynebacterium jeikeium*	colonized	Sig	Antibiogram
(Arnow et al., 1991)	*A. flavus & A. fumigatus*	Infected	Sig	Criteria *
(Döring et al., 1993)	*P. aeruginosa*	colonized	Sig	Antibiogram & Southern method
(Alberti et al., 2001)	*Invasive aspergillosis*	Infected	Sig	Criteria **
(Sexton et al., 2006)	*MRSA*	Infected	Sig	PFGE
(Talon et al., 2008)	*MRSA*	colonized	Sig	Antibiogram
(Munoz-Price et al., 2013)	*A. baumannii*	Infected	Sig	PFGE
(Muzslay et al., 2013b)	*E. faecalis (VSE)*	Infected	Sig	Antibiogram & PFGE
(Ao et al., 2014)	*Aspergillus spp*	Infected	Sig	PCR technique (RAPD Assay)
(Bonnal et al., 2015)	*Aspergillus spp*	Infected	Sig	Microsatellite markers & Southern method
(Cheng et al., 2015)	*zygomycosis*	Infected	Sig	PCR technique
(Shimose et al., 2016)	*Acinetobacter baumannii*	colonized	Sig	PFGE
(Kim et al., 2016b)	*MERS coronavirus*	Infected	Sig	PCR technique
(Haig et al., 2016)	*Pneumocystis carinii*	Infected	fluctuation	PCR technique PCR
(Carvalho et al., 2007)	*Invasive aspergillosis*	Infected	NS	Antibiogram

Sig= significance, NS= non-significance, PFGE= Pulsed-field gel electrophoresis and PCR= Polymerase chain reaction.

Routine nursing care is also important as it contributes to the airborne pathogen load. Previous work illustrates that increased activity in the hospital bay (e.g. patient washing that occurred behind closed curtains) is correlated with increased concentrations of bioaerosols, whereas sedentary

visitors did not (Hathway et al., 2013). Wards are generally full of moving patients, healthcare workers and visitors, leading to a possible re-contamination of the environment (Hardy et al., 2007; La Fauci et al., 2017), see (Table 2:4). Despite the fact that there are several papers demonstrating the effect of nursing activities on releasing or dispersing microorganisms, these typically occur during a sampling snapshot, and there is little data on the influence of these activities on the dispersion and deposition of microorganisms over time.

Housekeeping activities are essential for maintaining a clean environment and to reduce the number of microorganisms both in the air and on surfaces. However, some housekeeping activities have an impact on the environmental bioburden. Several studies show that floor cleaning (dry, spray, moist and wet mopping) has a significant effect on the level of airborne pathogens (Schmidt et al., 1984; Andersen et al., 2009; Park et al., 2013; La Fauci et al., 2017). Hand washing can be associated with bioaerosols, with a study showing a correlation with microorganisms in the air when the sink drain had more than 105 cfu/ml of *P. aeruginosa* (Döring et al., 1993). Healthcare laundry-related activities have been implicated, with one study associating this with a zygomycosis outbreak (Cheng et al., 2015). Waste disposal sites can also be a source of contamination that leads to aerosolisation (Verani et al., 2014). On the other hand, the removal of contaminated filters and cleaning/disinfection of air conditioning systems results in improvements in the indoor environments of the hospital with a lower level of airborne microorganisms (Arnow et al., 1991; Liguori et al., 2010). As a result, different levels of housekeeping activities that influence the microorganisms load raise the need for conducting more specific research to investigate their correlation with hygiene and environmental contamination. Also, there is a need for predictive models that can indicate when the cleaning and housekeeping activities should be performed.

Table 2:4 Summary of studies showing the effect of staff activities on the level of the airborne microorganism.

Reference	Type activities	Type of microbes or disease	P-value
(Humphreys et al., 1996; Moore et al., 2002)	physiotherapy cystic fibrosis patient	Burkholderia Cepacia	Sig
(Shiomori et al., 2002)	bedmaking	MRSA	Sig
(Lee et al., 2012)	showering	Aspergillus spp	Sig
(Hathway et al., 2013)	Patient Washing, curtain movement	ACC	Sig
(Arnow et al., 1991; Hsueh et al., 2014)	Filters cleaning	A. fumigatus	Sig
(Wong et al., 2011; Tekİn et al., 2013; Barbut et al., 2013)	Air disinfectant	ACC, MRSA, A. baumannii & TF	Sig
(Andersen et al., 2009)	Floor cleaning	ACC	Sig
(Schmidt et al., 1984)	Floor cleaning	ACC	NS

Sig= significance, NS= non-significance, ACC= airborne microbial concentration measured as aerobic colony count.

2.3.4.2 External sources

The relationship between microorganisms in the air within the hospital and those in the outside air is not yet clear. Two studies found that the external microbial load correlates with that of the indoor hospital environment (Tambekar et al., 2007; Ao et al., 2014), but another study in India (Nandalal and Somashekar, 2007) found no correlation between outdoor and indoor airborne microorganisms load in different places of the hospital. There is very limited data on other factors and environmental conditions in these studies; hence there is a need for more studies to assess this relationship. This factor will vary depending on the location of the hospital, how the room is connected to the rest of the building, and the wind direction.

2.3.5 Influence of environmental parameters

2.3.5.1 Relationship to indoor air quality (IAQ) parameters

IAQ parameters such as temperature, relative humidity, CO_2 level, particle mass concentration and particle size are important for the health and wellbeing of those in hospitals and may also influence the bioburden in the environment. Ambient air temperature and relative humidity are usually

measured in indoor environments to understand the thermal comfort and well-being of occupants. However, both parameters are also linked with the survival of microorganisms, with humidity being a particular concern. CO_2 is related to the exhaled breath of occupants and is frequently measured in indoor environments as an indicator of ventilation rates and occupancy.

Although the correlation between IAQ parameters and microorganism prevalence and survival has been studied for decades, there are conflicting results (Augustowska and Dutkiewicz, 2006; Hsu et al., 2012; Hathway et al., 2013; Mirhoseini et al., 2015; Huang et al., 2017; Osman et al., 2018; Božić et al., 2019) and it is not clear which parameters may be significant and how they interact together. If there are significant and consistent relationships between the microbial load in the air and IAQ parameters, this could allow IAQ to be used as a proxy for evaluating the likelihood of microorganisms being present in the air. The result of these parameters and airborne microorganisms are quantitative measurements. This is considered in greater detail in chapter 3, where a meta-analysis was performed to quantitatively investigate the relationships between the level of airborne microorganisms and IAQ parameters in a hospital environment.

2.3.6 Temporal factors (season, sampling time of the day)

Seasonal variations in airborne microorganisms have been recorded in this review, and there are differences in assessing the seasonal-related significance. Ten studies found that there were significant differences, while nine studies found that there were either none or fluctuating differences. More studies found higher bioburden during spring and summer, while lower levels were found mostly in winter (Table 2:5). There is also a conflict about significance in dry and rainy seasons (Niaré-Doumbo et al., 2014; Martínez-herrera et al., 2016). These conflicts can be justified by the sampling time (Martins-Diniz et al., 2005; Hathway et al., 2013) since they even showed different patterns when sampled over two consecutive days (Roberts et al., 2008; Hathway et al., 2013). *S. aureus* counts fluctuate across different places in hospitals within the same season (Nandalal and Somashekar, 2007). There are still doubts about the impact of seasons on the bioaerosol level, and the available data is not sufficient to measure the interaction of seasons with other environmental factors. It is also useful to record the

season in terms of temperature, relative humidity, rain and wind speed in order to obtain more reliable and internationally unified factors in assessing the impact of the airborne load.

Sampling time can be anytime in the morning, afternoon, evening or at night. Most papers have conducted sampling from 8:00 am to 5:00 pm, while only a few studies extended the hours of sampling to reach midnight. There is a significant difference between sampling time and the concentration of airborne load (Döring et al., 1993; Al-Shahwani, 2005; Augustowska and Dutkiewicz, 2006; Creamer et al., 2014; Fekadu and Getachewu, 2015). This correlates with activities and density of people that are usually higher in the morning (Rainer et al., 2001; Shiomori et al., 2002; Crimi et al., 2009; Hathway et al., 2013). Other papers found no significant impact of sampling at different times of the day (Ríos-Yuil et al., 2012; Killingley et al., 2016).

Table 2:5 Summary of studies showing the effect of season on the level of the airborne microorganism.

Reference	Country	Type of microbes	Season	Significant	High value	Low value
(Martins-diniz et al., 2005)	Brazil	*Aspergillus spp*		sig	March-morning	
		Fusarium spp	3 Autumn-Summer	sig	March-morning	
		Cladophialophora spp		Sig	March-afternoon	
(Brunetti et al., 2006)	Italy	TF	4	sig	Spring and Summer	Winter and Autumn
(Augustowska and Dutkiewicz, 2006)	Poland	ACC & TF	4	sig	Spring and Summer	Winter
(Lee et al., 2007)	USA	*Aspergillus spp*	3 Spring-Autumn	sig	Summer	Spring
(Park et al., 2013)	Korea	ACC & TF	3 Summer-Winter	sig	Summer	Winter
(Cavallo et al., 2013)	Italy	*Aspergillus spp*	4	sig		
(Ao et al., 2014)	China	*Aspergillus spp*	4	sig	Spring and Summer	Winter
(Sabino et al., 2014)	Portugal	*Aspergillus spp*	4	sig	Spring and Summer	Winter
(Gheith et al., 2015)	Tunisia	TF	4	sig	Summer and Autumn	
		Aspergillus spp	4	NS		
(Hospenthal et al., 1998)	USA	*conidia*	4	NS		
(Panagopoulou et al., 2002)	Greece	TF	4	NS		

Reference	Country	Type of microbes	Season	Significant	High value	Low value
(Cordeiro et al., 2010)	Brazil	TF	4	NS		
(Farmaki et al., 2007)	Greece	TF	4	fluctuations	Summer and Autumn	Winter
(Nandalal and Somashekar, 2007)	India	*S. aureus*	4	fluctuations		
(Falvey and Streifel, 2007)	USA	*A. fumigatus*	4	fluctuations		
(Rainer et al., 2001)	Austria	*Cladosporium spp* *other Fungi spp*	4 4	fluctuations no fluctuations	Winter-Summer	
(Niaré-Doumbo et al., 2014)	Mali	TF	dry (Mar) rainy (Sep)	sig	Autumn	Spring
(Martínez-herrera et al., 2016)	Mexico	TF	dry (Nov-Apr) rainy (May-Oct)	NS		

Sig= significance, NS= non-significance, ACC= airborne microbial concentration measured as aerobic colony count, TF= total fungal.

2.3.7 Spatio-geographical factors (country, hospital, units, location inside the room or ward) other factors

The results show that the UK has the highest percentage (18%) of papers that perform air sampling in different units and locations around the hospital. See (Table 2:5).

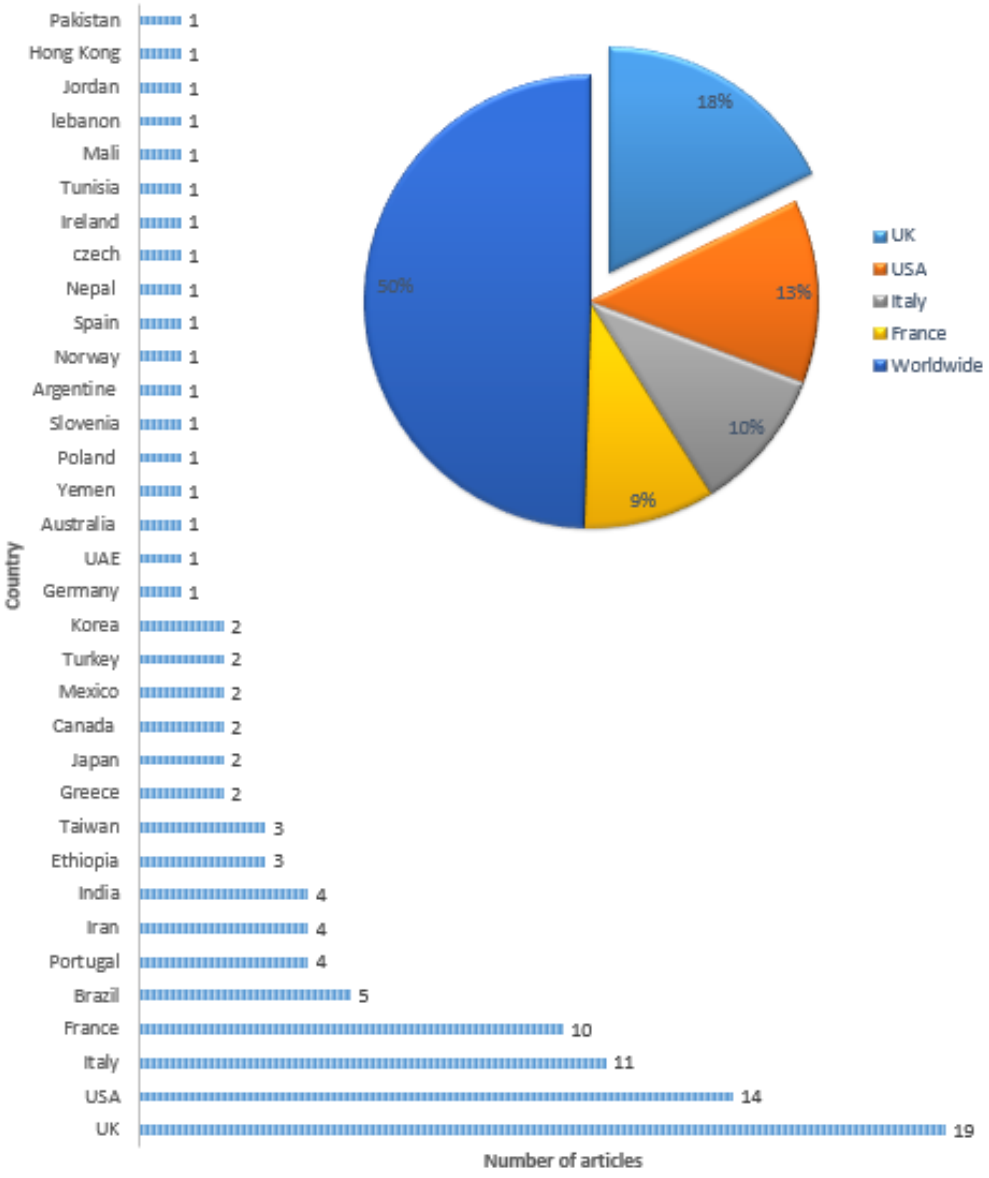

Figure 2:5 Distribution of articles per country

Hospitals have many departments and units in which air sampling was conducted. Many papers have studied microbial concentrations in different locations and found that there is a significant difference between the level of airborne microorganisms and different hospitals in the same country (Farmaki et al., 2007). Several studies found that there is a significant difference between bioburden and different departments or units within the same hospital with regards to different types of microorganism (Jaffal et al., 1997; Al-Shahwani, 2005; Nandalal and Somashekar, 2007; Lee et al., 2012; Gaudart et al., 2013; Azimi et al., 2013; Méheust et al., 2014; Gizaw et al., 2016; Martínez-herrera et al., 2016). Furthermore, different locations inside the same unit were found to have significant differences between airborne bioburden. According to Matoušková and Holy (2014), the entrance of a transplant unit had higher ACC concentrations compared with the centre of the unit. One study found more *Aspergillus spp* concentration in a shower area than in the centre of the room (Picot-Guéraud et al., 2015). This is confirmed by another study that measured the concentration of bacteria in toilet areas (Gizaw et al., 2016).

Other findings contradict this and suggest that there is no significant difference between different locations. Previous work found no difference in the level of airborne microorganisms in different hospitals in Greece (Panagopoulou et al., 2002). A study on ACC and three studies on TF found no significant difference in the level of airborne microorganisms in different areas of the same hospital (Panagopoulou et al., 2002; Saadoun et al., 2015; Mirhoseini et al., 2015; Fekadu and Getachewu, 2015). Others found no significant association between different locations inside the room or unit and the level of airborne microorganisms. The level of TF was found to be similar inside intensive care unit (ICU) and in the corridors (Rainer et al., 2001), while the level of *Aspergillus spp* was similar in a patient's room and shower room (Lee et al., 2007). A study on the percentage of microorganisms and location of colonised patients in the ICU also found no significant association (Barbolla et al., 2008). Another study that took samples at 0.5-3m from patients' heads supports the same claim that there is no significant association (Gehanno et al., 2009). Finally, the two studies concerning the measles virus and influenza virus found no significant

association between viral load in the air within the patient room and distance from the patient (Mirhoseini et al., 2015; Killingley et al., 2016).

It is clear that there are conflicting results in many papers that have studied airborne microorganism concentrations in hospitals, which can be due to many reasons. Microorganism loads can vary in different units of the same hospital, especially when sampled at different times, with temperature and humidity being a factor that affects their concentration. The presence of water can be a significant factor that leads to higher concentrations of microorganisms through re-aerosolization (Jaffal et al., 1997; Lee et al., 2012; Gizaw et al., 2016).

2.4 Summary

This chapter systematically reviewed studies that sampled airborne microorganisms in hospital wards and shows that there are several gaps in the literature. This research addresses the following gaps:

- There are no guidelines for air sampling to guarantee consistent results. Using settle plates to assess airborne risk offers a cheap and easy sampling approach, but it is necessary to carefully consider how to use sampling results to determine airborne concentrations (addressed in chapter 2).
- The relationships between airborne microorganisms and IAQ parameters (temperature, relative humidity, CO_2, particle mass concentration and particulate matter of size) have been studied for decades. There are however conflicting results, and it is not clear which parameters are significant and how they interact with each other. The availability of quantitative studies in the literature makes it possible to perform a meta-analysis study to investigate the relationships between the level of airborne microorganisms and IAQ parameters in a hospital environment and to formally assess the strength of relationships between parameters (addressed in chapter 3).
- There are only a small number of studies that present quantitative data while measuring the environment and activity factors that affect the presence and quantity of airborne microorganisms. This may mean that

for some microorganisms and influencing factors that there is not enough data to make a good judgement on the importance of different factors (addressed in chapters 4 and 5).

- There is a lack of evidence on the effect of the ventilation rate on the deposition of microorganisms and hence the surface transmission risk (addressed in chapters 6 and 7).

3 The relationship between indoor air quality parameters and airborne microorganisms in hospital environments.

3.1 Introduction

This chapter builds on the systematic review presented in chapter 2, with a more in-depth focus on the relationships between indoor air quality and airborne microorganisms. A meta-analysis is carried out to provide a quantitative analysis of the relationships between the level of airborne microorganisms and IAQ parameters in a hospital environment. By bringing together data from multiple studies, the chapter aims to formally assess the strength of relationships between parameters, and to determine where there are gaps in data that could inform future experimental studies in healthcare settings. This chapter investigates whether there are quantitative relationships between the concentration of airborne microorganisms and the IAQ in the hospital environment. This study can also inform new predictive models that provide an improved method for monitoring the concentration of airborne microorganisms in real-time through measurement of IAQ parameters.

3.2 Methodology

3.2.1 Search and inclusion criteria

A systematic review was performed to identify relevant studies. This used the same search strategy and broad exclusion criteria as in Chapter 2: Full-text articles published in English that include air sampling data for microorganisms and IAQ parameters in patient areas of hospitals were selected for inclusion. The reference lists of all selected studies were screened to identify other likely eligible studies. Studies with relevant data were included for the meta-analysis (Figure 3:1); studies had to present quantitative data on the airborne microbial concentration measured as aerobic colony count (ACC) or airborne total fungi (TF) with at least one IAQ factor: temperature, relative humidity, CO_2, particle mass concentration (≤5 or >5 µg/m^3) or particulate matter of size (≤5 or >5 µm) measured at the

same time point. The directed acyclic graph (DAG) approach is a good way to investigate causality with variables with respect to confounding as it helps in understanding the consequences of conditioning to different factors. The DAGitty and statistical software R 4.0.0 (package ‘ggdag’ version 0.2.3) were used to build a directed acyclic graph (Figure 3:2) to describe how potential confounders and the air quality parameters relate to microbial measures (Textor et al., 2017).

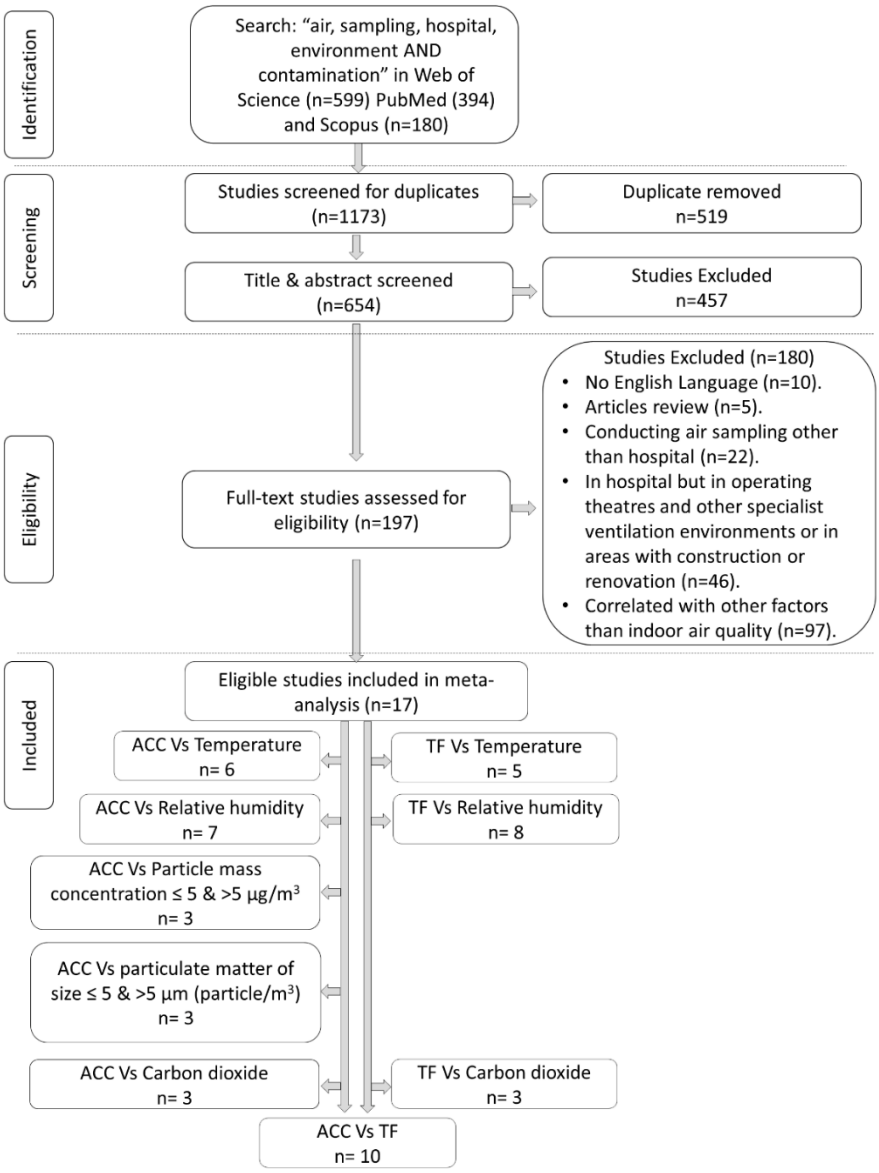

Figure 3:1 Flow chart of the systematic review and meta-analysis phases, search strategy and exclusion criteria. ACC: airborne microbial concentration measured as aerobic colony count and TF: airborne total fungi.

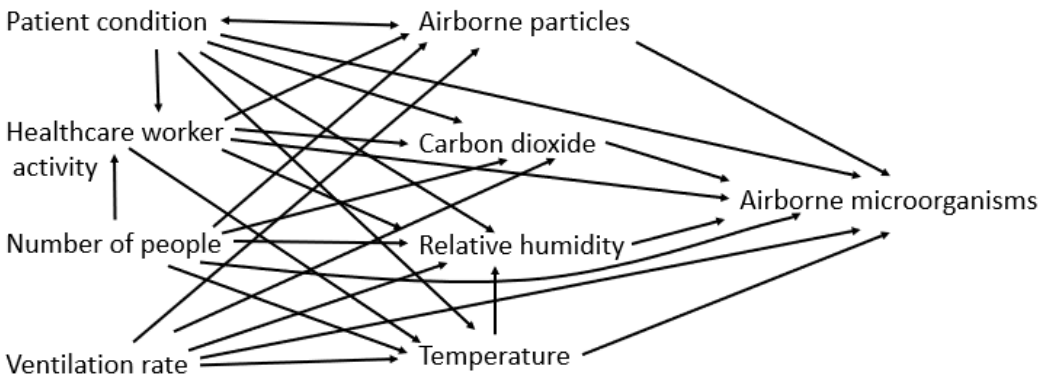

Figure 3:2 Directed acyclic graph showing IAQ parameters that may affect the bioburden in the air and potential confounding factors.

3.2.2 Data extraction and quality appraisal

All corresponding authors for included studies were contacted for raw data where the data available within the paper was not sufficient to conduct analysis. Correlation coefficient and sample size were extracted directly from the study, derived from graphed points, obtained from tabulated values or calculated from raw data, which were provided by the corresponding author via private correspondence. Equation (3.1) was used to compute the correlation coefficient from multiple regression and the general linear model for taking covariates into account (Nakagawa and Cuthill, 2007).

$$r = \frac{t}{\sqrt{t^2 + df}} \tag{3.1}$$

Where df is the degrees of freedom used for a corresponding t value in a linear model. Outliers and influential observations are very likely to weaken the validity and robustness of the conclusions from a meta-analysis (Viechtbauer, 2010). Sensitivity analysis of the meta-analyses to detect potentially outlying studies was performed using visual approaches including (1) externally standardised residuals, (2) Difference in Fits (DFFITS) values, (3) Cook's distances, (4) covariance ratios, (5) leave-one-out estimates of the amount of heterogeneity, (6) leave-one-out values of the test statistics for heterogeneity, (7) hat values, and (8) weights (Viechtbauer and Cheung, 2010). If observations were beyond the lower and upper limit of DFFITS, they were excluded from the meta-analysis, as their inclusion could lead to notable changes in the pooled (overall) estimate effect size of meta-analysis.

To test heterogeneity between-studies, the Q statistic was used to examine the null hypothesis that all studies had the same true effect: $\tau 2=0$ (Hedges, 1982). The 95% CI around the I^2 statistic was also calculated to determine the level of heterogeneity present.

The meta-analysis was based on a Fisher Z transformation of the correlation coefficient to obtain weightings for each study. Fisher transformed correlations are always less biased than when untransformed correlations are used (Rosenbaum et al., 1987). A random-effect meta-analysis model is used since the studies came from different populations and included design-related heterogeneity. Random effects models are more appropriate since the aim is to generalise beyond the studies included in the meta-analysis (Hunter and Schmidt, 2000; Field, 2003). Forest plots were used to visualise the overall estimates of the study effects with corresponding confidence intervals (Lewis and Clarke, 2001). This systematic review and meta-analysis was performed according to the Preferred Reporting Items for the Systematic Reviews and Meta-Analysis (PRISMA) guidance (Liberati et al., 2009; Moher et al., 2009). The statistical software R 4.0.0 (package ‘meta’ version 4.12-0 and package ‘metacor’ version 1.0-2.1) was used to perform the meta-analysis.

3.3 Results and discussion

To the best of our knowledge this is the first systematic review and meta-analysis to quantitatively examine the relationships between microbes in air and IAQ parameters in hospital environments. A total of 1173 studies were retrieved, 654 studies screened after duplicates were removed. After screening through titles and abstracts, 197 studies remained for full text assessed for eligibility. The majority of studies considered bacteria and/or fungi, and no studies had sufficient data to assess correlations between virus in air and the IAQ parameters in a hospital setting. Seventeen studies were included in the final meta-analysis (Figure 3:1). These presented quantitative airborne microbial concentration measured as aerobic colony count (ACC) or airborne total fungi concentration (TF) with at least one quantitative factor of the IAQ parameters at the same time point in a hospital

setting and the correlation coefficient values and sample size for the relationships are given for each study (Table 3:1) (Jaffal et al., 1997; Augustowska and Dutkiewicz, 2006; Hsu et al., 2012; Tekİn et al., 2013; Hathway et al., 2013; Huang et al., 2013; Azimi et al., 2013; Park et al., 2013; Méheust et al., 2013a; Yang et al., 2014; Mirhoseini et al., 2015; Fekadu and Getachewu, 2015; Sajjadi et al., 2016; Huang et al., 2017; Demirel et al., 2017; Osman et al., 2018; Božić et al., 2019).

Although the importance of ensuring good IAQ to minimise airborne microorganism transmission is recognised (Morawska et al., 2020b), we found that there are a very small number of studies that carry out sufficient quantitative measurement to reliably assess relationships between airborne microorganisms and environmental parameters.

The forest plots prepared were for the Fisher Z-transformed correlation which was used to test the hypotheses about the value of the correlation coefficient. In order to interpret the results, the transformed values of pooled correlations were converted back to the original metric in the text. The studies were checked for the presence of outliers and influential observations that might bias the results, but none was detected. The heterogeneity was not statistically significant and I^2 was very low between most studies. The correlations between ACC or TF and IAQ are as shown below. Figure 3:2(Rohrer, 2018) Uncorrelated measurement error cannot be elucidated from the articles, so we assume similar bands of error and thus do not include it in the statistical analysis.

Table 3:1 Studies included in the meta-analysis.

Study	Country	Place	sampling daytime	Season	Correlation ACC (cfu/m3) VS…								Correlation TF (cfu/m3) VS…		
					Temp. (°C)	RH (%)	PM≤ 5 (µg/m^3)	PM> 5 (µg/m^3)	PS≤ 5 (µm)	PS> 5 (µm)	CO_2 (ppm)	TF (cfu/m^3)	Temp. (°C)	RH (%)	CO2 (ppm)
Božić et al (2019) (Božić et al., 2019)	Bosnia and Herzegovina	Different clinics*	N/A	Feb-Mar		r=0.22 *p*=0.21 n=35						r=0.11 *p*=0.54 n=35		r=0.30 *p*=0.08 n=35	
Osman et al (2018) (Osman et al., 2018)	Egypt	ICU**	Morning and afternoon	Year-round	r=-0.40 p<0.05 n=24	r=-0.63 p<0.05 n=24							r=-0.29 p<0.05 n=24	r=-0.43 p<0.05 n=24	
Huang et al (2017) (Huang et al., 2017)	Taiwan	Different clinics in different hospitals**	N/A	Oct-Feb			r=0.53 *p<0.01* n=70	r=0.43 *p<0.01* n=70	r=0.39 *p<0.05* n=70	r=0.39 *p<0.05* n=70					
Demirel et al (2017) (Demirel et al., 2017)	Turkey	Neonatal ICU**	N/A	Year-round										r=-0.24 *p<0.01* n=103	
Sajjadi et al (2016) (Sajjadi et al., 2016)	Iran	Waiting hall emergency ward*	Morning and afternoon	N/A	rho=0.43 *p*<0.01 n=28	rho=-0.08 *p*>0.05 n=28						r=0.48 *p*<0.01 n=96	rho=0.22 *p*>0.05 n=28	rho=0.29 *p*<0.05 n=28	
Mirhoseini et al (2015) (Mirhoseini et al., 2015)	Iran	ICU**	N/A	N/A	r=0.02 *p*>0.05 n=80	r=0.33 *p*<0.05 n=80			rho=0.76 *p*<0.01 n=80	rho=0.37 *p*>0.05 n=80					
Fekadu and Getachewu (2015) (Fekadu and Getachewu, 2015)	Ethiopia	Two maternity wards*	Morning and evening	Feb - June								r=0.72 *p*<0.01 n=42			
Study	Country	Place	sampling daytime	Season	Correlation ACC (cfu/m3) VS…								Correlation TF (cfu/m3) VS…		

					Temp. (°C)	RH (%)	PM≤ 5 (µg/m^3)	PM> 5 (µg/m^3)	PS≤ 5 (µm)	PS> 5 (µm)	CO_2 (ppm)	TF (cfu/m^3)	Temp. (°C)	RH (%)	CO2 (ppm)
Yang et al (2014) (Yang et al., 2014)	Taiwan	Different units*	Morning and evening	June	r=0.04 *p*=0.72 n=80	r=0.12 *p*=0.28 n=80	r=0.59 *p*=NM n=11	r=0.01 *p*=NM n=11			r=0.59 *p*=NM n=11	r=0.30 *p*=0.59 n=80	r=0.12 *p*=0.30 n=80	r=0.32 *p<0.01* n=80	r=0.01 *p*=NM n=11
Tekİn et al (2013) (Tekİn et al., 2013)	Turkey	Burn centre & clinical microbiology laboratory*	N/A	N/A								r=0.29 *p*=0.35 n=12			
Park et al (2013) (Park et al., 2013)	Korea	lobbies**	Morning, afternoon and evening	Year-round	r=0.43 *p*<0.01 n=76	r=0.10 *p*<0.25 n=76					r=0.58 *p*<0.001 n=76	r=-0.19 *p*=0.52 n=14		r=0.17 *p*<0.01 n=76	r=0.02 *p*=0.056 n=76
Méheust et al (2013) (Me´heust et al., 2013)	France	Laboratory room*	N/A	N/A								r=-0.85 *p*=0.06 n=5			
Huang et al (2013)(Huang et al., 2013)	Taiwan	Two ICU*	N/A	Aug-Oct								r=0.95 p=0.05 n= 4			
Hathway et al (2013) (Hathway et al., 2013)	United Kingdom	A respiratory ward*	Morning and evening	Aug					rho=0.27 *p*=0.6 n=48	rho=0.80 *p<0.01* n=48					
Azimi et al (2013) (Azimi et al., 2013)	Iran	Nursing Stations*	N/A	Jan - Apr									r=-0.10 *p*=0.78 n=10	r=0.23 *p*=0.52 n=10	
Hsu et al (2012) (Hsu et al., 2012)	Taiwan	Different units**	Morning and afternoon	N/A			r=0.12 *p*=NM n=60	r=0.08 *p*=NM n=60			r=0.44 *p=NM* n=60				r=-0.16 *p*=NM n=60
Study	Country	Place	sampling daytime	Season	Correlation ACC (cfu/m3) VS…								Correlation TF (cfu/m3) VS…		

					Temp. (°C)	RH (%)	PM≤ 5 (µg/m^3)	PM> 5 (µg/m^3)	PS≤ 5 (µm)	PS> 5 (µm)	CO_2 (ppm)	TF (cfu/m^3)	Temp. (°C)	RH (%)	CO2 (ppm)
Augustowska and Dutkiewicz (2006) (Augustowska and Dutkiewicz, 2006)	Poland	Wards of the pneumological department*	Morning and afternoon	Jan - May	r=0.31 *p*=0.33 n=12	r=0.28 *p*=0.38 n=12						r=0.29 *p*=0.35 n=12	r=-0.12 *p*=0.71 n=12	r=-0.17 *p*=0.60 n=12	
Jaffal et al (1997) (Jaffal et al., 1997)	United Arab Emirates	Different units *	N/A	N/A								r=-0.21 *p*=0.73 n=5			

ACC = airborne microbial concentration measured as aerobic colony count, TF = airborne total fungi, Temp= Temperature, Rh= Relative humidity, PM= particle mass concentration, PS= particulate matter of size, r= Pearson correlation coefficient, rho = Spearman's rank correlation coefficient, n= Sample size, N/A= Not available, * study conducted in hospital, ** Study conducted in more than one hospital.

3.3.1 Sampling approaches

As discussed in section 2 (2.3.2) there are two main approaches used in air sampling studies: active sampling and passive sampling. Most of the studies identified in the review used active sampling (Table 3:2) for the benefits that it offers, including that it is fast and not dependent on the local room airflow pattern. Also, active sampling can provide similar results with some differences depending on the type, air flow rate and manufacturer of the device (Haig et al., 2016). However, results from papers using different types of active samplers can still be used in comparisons and can still provide useful information. Some studies use more inexpensive passive sampling using an open petri dish. Airborne microorganisms that are not removed by ventilation may eventually deposit onto surfaces, and the numbers that deposit are expected to correlate with the number of microorganisms present in the air.

In order for passive sampling to provide more meaningful results, mathematical equations are required to calculate the deposition rate in terms of cfu/m^3 (Hsu et al., 2012; Fekadu and Getachewu, 2015). The duration of samples and the interval between them are contributing factors that affect the results and that need to be taken into consideration when performing analyses and comparisons. A previous study shows fluctuation of airborne microbial concentrations with time in the same location, with intervals of 15 minutes and duration of 5 minutes each over 8 hours of sampling (Hathway et al., 2013). The summary of results in Table 3:2, show that most of the studies present their findings based on a snapshot air sampling rather than intensively performing multiple samples over a long time. This can lead to misleading conclusions as the results may be too few to reflect the accurate correlations between the microbial sample and the environmental conditions.

Table 3:2 A summary of air sampling approaches in the literature.

Study	Air sampler	Manufacturer	Airflow (l/min)	Sampling duration (min)	Total volume (l)	Interval between samples (per day)	ACC Media	TF Media
Božić et al (2019) (Božić et al., 2019)	Sampl'air™ Lite	BioMérieux, , France	200	N/A	N/A	3-4	TSA	SDA
Osman et al (2018) (Osman et al., 2018)	Andersen two-stage viable cascade	Tisch Environmental Cleves, OH, USA	28.3	5	141.5	4	Nutrient agar (with cyclo- heximide)	Rose-Bengal streptomycin agar
Huang et al (2017) (Huang et al., 2017)	MAS-100	Merck Inc., USA	100	N/A	N/A	2	TSA	N/A
Demirel et al (2017) (Demirel et al., 2017)	MAS-100 M Air-T Air IDEAL	Merck Inc Millipore Biomerieux	N/A	N/A	100	1	N/A	Dichloran 18% glycerol agar
Sajjadi et al (2016) (Sajjadi et al., 2016)	Air sampling pump	SKC	61	5	305	2	TSA (with nystatin)	SDA (with chloramphenicol antibiotic)
Mirhoseini et al (2015) (Mirhoseini et al., 2015)	Glass impingers (AGI)	N/A	12.5	180-240	2250-3000	N/A	20 ml of phosphate buffer then TSA	N/A
Fekadu and Getachewu (2015) (Fekadu and Getachewu, 2015)	Passive sampling (9cm diameter Petri dishes)	N/A	N/A	N/A	N/A	2	2% nutrient agar	4% SDA
Yang et al (2014) (Yang et al., 2014)	single-stage impactor	Standard BioStage, SKC Inc., PA, USA	28.3	2	56.6	2	TSA	MEA
Tekİn et al (2013) (Tekİn et al., 2013)	Air Test Omega	LCB, France	N/A	N/A	N/A	2	plate count agar (PCA)	SDA
Park et al (2013) (Park et al., 2013)	Anderson single-stage cascade	N/A	28.3	5	141.5	3	TSA	SDA (with chloramphenicol)

Study	Air sampler	Manufacturer	Airflow (l/min)	Sampling duration (min)	Total volume (l)	Interval between samples (per day)	ACC Media	TF Media
Méheust et al (2013) (Me´heust et al., 2013)	Sampl'Air Air Ideal AirPort MD8/BACTair	AES Chemunex, France bioM´erieux, France Sartorius, France	100 100 125	1-10	100-1000	5	TSA	SDA
Huang et al (2013)(Huang et al., 2013)	single-stage bioaerosol impactor	Standard BioStage, SKC Inc., PA, USA	28.3	2	56.6	2	TSA	MEA
Hathway et al (2013) (Hathway et al., 2013)	Microbio MB2	Fred Parrett, UK	500	5	2500	32 (Every 15 min)	TSA	N/A
Azimi et al (2013) (Azimi et al., 2013)	Andersen one- stage viable single-stage viable cascade	Quick Take-30, SKC, USA SKC BioStage single-stage viable cascade impactors	28.3	2	56.6	N/A	N/A	SDA
Hsu et al (2012) (Hsu et al., 2012)	Passive sampling (open Petri dishes)	N/A	N/A	N/A	N/A	2	Luria broth agar	N/A
Augustowska and Dutkiewicz (2006) (Augustowska and Dutkiewicz, 2006)	custom-designed particle-sizing slit sampler	N/A	20	N/A	N/A	4	N/A	SDA
Jaffal et al (1997) (Jaffal et al., 1997)	One stage culture plate impactor MK2	Casella, London	30	5	150	N/A	Blood agar	SDA

ACC = Airborne microbial concentration measured as aerobic colony count, MEA=Malt extract agar, SDA=Sabouraud dextrose agar, TF = Airborne total fungi, TSA= Tryptic soy agar

3.3.2 Correlation between airborne microorganisms with ambient air temperature and ambient relative humidity.

Six studies provided quantitative data to assess the relationships between the concentration of airborne microorganisms and temperature within the hospital environment. Temperatures recorded within the studies ranged from 17.4°C to 27°C, for measured microbial concentration of ACC 50-6295 cfu/m^3 and TF 4-1125 cfu/m^3. As shown in (Figure 3:3 **a**), ACC was weakly positively correlated with temperature (r=0.25 [95% CI=0.06-0.42], *P*=0.01) with a sample size ranging from 12-80 with a total of 300 values over all studies (Augustowska and Dutkiewicz, 2006; Park et al., 2013; Yang et al., 2014; Mirhoseini et al., 2015; Sajjadi et al., 2016; Osman et al., 2018). TF was not significantly correlated with temperature (r=0.05 [95% CI=-0.12-0.21], *P*=0.60) with a sample size ranged from 12-80 and a total of 154 values (Figure 3:3 **b**) (Augustowska and Dutkiewicz, 2006; Azimi et al., 2013; Yang et al., 2014; Sajjadi et al., 2016; Osman et al., 2018).

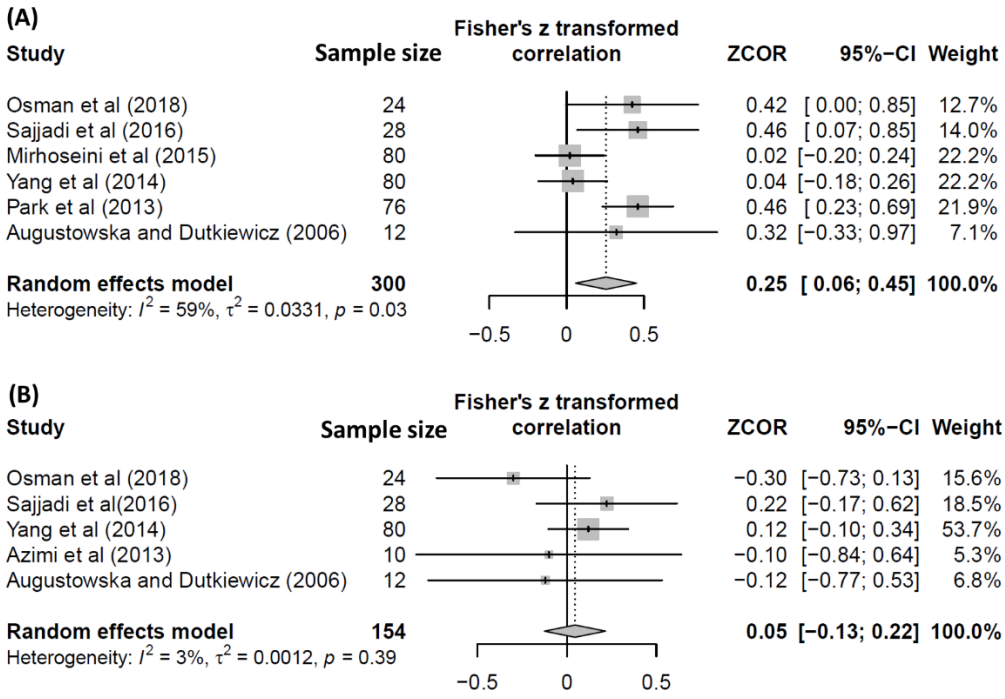

Figure 3:3 Forest plot showing the relationship between temperature and microorganism concentrations using Fisher's transformed correlation. (a)

correlation with airborne microbial concentration measured as aerobic colony count. (b) correlation with airborne total fungi concentration.

The relative humidity was measured in eight studies and the data was provided alongside microbial concentrations in air. It was reported in the studies ranged from 17% to 79% with microbial concentrations ACC in the range 20-6295 cfu/m^3 and TF 4-1125 cfu/m^3. Relative humidity was not significantly correlated with ACC r=0.06 (95% CI=-0.15-0.27), *P*= 0.59 with a sample size ranged from 12-80 and a total of 333 values (Figure 3:4 a) (Augustowska and Dutkiewicz, 2006; Park et al., 2013; Yang et al., 2014; Mirhoseini et al., 2015; Sajjadi et al., 2016; Osman et al., 2018; Božić et al., 2019). There was also no correlation with TF r=0.07 (95% CI=-0.16-0.28), P=0.56 with a sample size ranged from 12-103 and a total of 368 values (Figure 3:4 b) (Augustowska and Dutkiewicz, 2006; Azimi et al., 2013; Park et al., 2013; Yang et al., 2014; Sajjadi et al., 2016; Demirel et al., 2017; Osman et al., 2018; Božić et al., 2019).

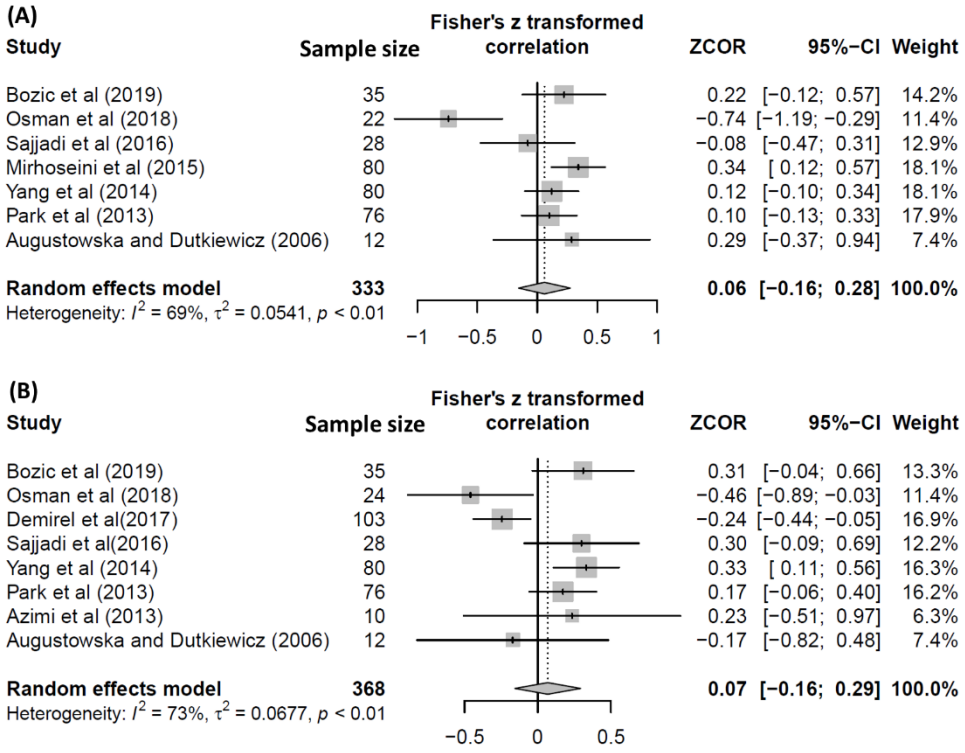

Figure 3:4 Forest plot showing the relationship between relative humidity and microorganism concentration using Fisher's transformed correlation. (a)

correlation with airborne microbial concentration measured as aerobic colony count. (b) correlation with airborne total fungi concentration.

The meta-analysis suggests there is a significant positive relationship between airborne bacteria concentration and temperature, while there was no statistically significant relationship between airborne microbial concentration and relative humidity. For the airborne fungi concentration, the correlation with both temperature and relative humidity were not found to be significant. The Pooled effect estimate is low, and the confidence intervals are wide meaning that confidence in the relationship between microorganisms and temperature and humidity are low. This makes physical sense as most microorganisms favour warmer conditions for faster replication although many survive well at cooler room temperatures. A previous study has found that increasing the temperature from 15°C to 25°C and 34% and 75% influenced the survival rate of *Pseudomonas sp*., *Acinetobacter calcoaceticus*, *corynebacteria, Staphylococcus sp*., and *Staphylococcus aureus* on glass surfaces different depending on the type of microbes from no difference to slightly negative relationship (McEldowney and Fletcher, 1988). Another study found that there is no significant relationship between atmospheric temperature (16 °C & 24 °C) and survival rate of airborne Serratia marcescens, Escherichia coli, and Bacillus subtilis (Ehrlich et al., 1970).

Many bacteria and fungi favour higher humidity conditions but studies show that response of microorganisms to humidity is more complex; different species do not respond to relative humidity in the same way with regards to survival (Zoz et al., 2016). For example, the survival of Escherichia coli (Shigatoxin-producing) at a temperature of 20 °C and different relative humidity (44%, 70%, 85% and 98%) has a U-shape response where the lowest survival was at 85% RH (Møretrø et al., 2010). Several studies have also showed that viruses have a more complex response to humidity, with lipid envelope viruses surviving longer at low humidity (20-30% RH) while non-lipid enveloped viruses preferring higher humidity (70-90 %RH) (Tang, 2009). Some viruses also express a U-shaped response with the lowest survival at mid-range humidity (40-60% RH).(Tang, 2009) Humidity may also have a further effect where microorganisms are released into the air through

aerosolisation from a liquid, with lower humidity resulting in smaller aerosols which may be suspended for longer in the environment (Tang, 2009). Recommendations for temperature and humidity in hospitals vary by country, season, ventilation strategy and by clinical area of the hospital. Guidance for UK hospitals recommends 18-28 °C in ward areas, with 18-25 in most clinical spaces. No specific recommendations are given for humidity and it is rare that humidity is controlled (Department of Health / Estates and Facilities Division, 2007). In the USA, ASHRAE recommend 21-24 °C in patient rooms and also do not specify humidity control, however in clinical areas they typically recommend 30-60 % RH (Sheerin et al., 2020). Recommendations for patient rooms in Japan vary by season with temperature (24-27 °C) and humidity (50-60% RH) recommended for summer compared to winter (20-24 °C, 40-50% RH) (Tang, 2009). The lack of clear correlation between microbial load in the air and the temperature and humidity likely reflects the large range of microorganisms present in a hospital setting and their different responses to the environmental conditions. Further data that measures the prevalence of specific microbial species would help to understand how these relationships depend on the particular microorganism. The understanding of how temperature and humidity affects the evaporation of microbial aerosols also poses the question as to whether or not the greater temperature ranges and lower winter relative humidity (20%-35%) seen in naturally ventilated hospital environments in colder climates have higher suspension rate of microorganisms in air, lower deposition rates on surfaces, or lower survival rate of airborne pathogen than in spaces with a higher level of control through the building HVAC system. An in-depth study across a range of comparable environments would be necessary to answer this question.

3.3.3 Correlation between airborne microorganisms and CO_2

CO_2 is present within the exhaled breath of occupants, and hence the value indicates how much exhaled breath is retained in the room air. Only three studies provided sufficient quantitative data to evaluate the relationship between CO_2 and microorganism concentration. Within these studies the

reported CO2 concentration range was 470-1022 ppm above background, TF level 11 – 1400 cfu/m^3 and ACC level 50 – 3000 cfu/m^3. The sample size ranged from 11-76 with a total of 147 values across the three eligible studies (Hsu et al., 2012; Park et al., 2013; Yang et al., 2014). A moderately significant relationship was found for ACC r=0.53 (95% CI=0.40-0.64), P<0.001 (Figure 3:5 a) while TF was not significantly correlated with CO_2 level r=-0.06 (95% CI=-0.22-0.11), *P*=0.51 (Figure 3:5 b).

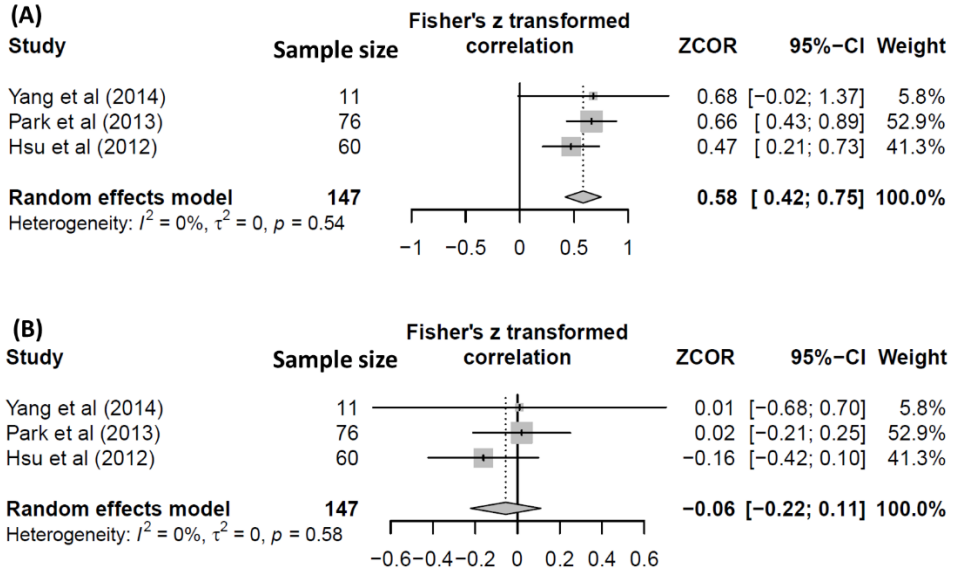

Figure 3:5 Forest plot showing the relationship between Carbon dioxide and microorganism concentration using Fisher's transformed correlation. (a) correlation with airborne microbial concentration measured as aerobic colony count. (b) correlation airborne with total fungi concentration.

The moderate and positive significant relationship between airborne bacteria concentration and CO_2 using a pooled estimate is intermediate, and confidence intervals are tight r=0.53 (95% CI= [0.40; 0.64]), P<0.001. This result highlights the likely importance of i) ventilation, which is the process of diluting, removing and replacing the air in a specific area naturally or mechanically, ii) the room occupancy which will contribute to bacterial generation through respiratory sources, natural skin shedding and activities such as bed making that may resuspend microorganisms (Park et al., 2013; Yang et al., 2014; Dougall et al., 2019).

Conversely, there was no relationship between total fungi concentration and CO_2 level. This result can be interpreted according to previous work that found people shed half the number of bacteria as fungi (Cundell, 2018). It is also likely that in many settings TF is influenced by the fungi in outdoor air and hence would only be influenced by ventilation is there is effective filtration in place (Tambekar et al., 2007). Studies have shown that the level of CO_2 level has a positive correlation with occupied rooms, room temperature and relative humidity (Al-Shahwani, 2005; Park et al., 2013; Yang et al., 2014). Although it is possible to estimate ventilation rates using exhaled CO_2 levels as a proxy, measuring the ventilation rate is not straightforward. Recommended ventilation rates in hospital wards vary worldwide and depend on the climate and ventilation approach. In the USA, ASHRAE recommend 6 ACH, however only 2 ACH is required to be fresh air and the remaining 4 ACH can be recirculated with appropriate filtration. UK hospitals recommend 6 ACH full fresh air, but do permit natural ventilation which will be variable (Department of Health / Estates and Facilities Division, 2007). Ventilation rates in many hospitals do not necessarily meet these standards and reflect the standards at the time of construction and the maintenance of the ventilation systems.

3.3.4 Correlation between airborne microorganisms and airborne particles

Three eligible studies considered the correlation with particle mass concentration, with values reported only for ACC and not TF. Across these studies the sample size ranged from 11-70, with a total of 141 measurements of airborne microorganism concentration (ACC ranged from 378-3000 cfu/m^3, particle mass ≤5 µg [5-61 µg/m3], and >5 µg [18.8-188 µg/m3]) (Hsu et al., 2012; Yang et al., 2014; Huang et al., 2017). There was a moderately significant correlation between ACC and particle mass concentration ≤5 µg/m3 r=0.40 (95% CI= [0.04; 0.66]), P= 0.03 (Figure 3:6 a), while ACC was not significantly correlated with particle mass concentration >5 µg/m3 r=0.23 (95% CI= [-0.07; 0.49]), P= 0.13 (Figure 3:6 b).

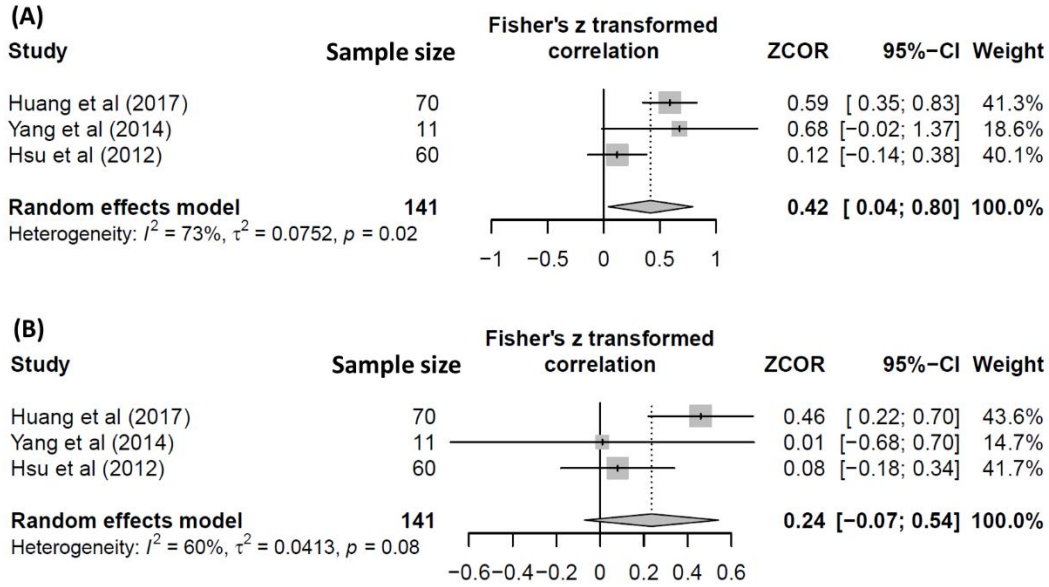

Figure 3:6 Forest plot for the studies reporting relationships between the particle mass concentration and airborne microbial concentration using Fisher's transformed correlation. (a) Correlation with particle mass concentration ≤5 µg/m^3. (b) Correlation with particle mass concentration >5 µg/m^3.

To evaluate correlations with particulate matter size, three studies provided data with a sample size ranging from 48-70 and a total of 198 values (ACC ranged from 50-650 cfu/m^3, particulate matter of size ≤5 µm ranged from 8 x 10^3 – 4 x 10^7 particle/m3, and >5 µm from 1 x 10^3 – 1.1 x 10^5 particle/m^3). There was a moderately significant correlation between ACC and particulate matter of size ≤5 and >5 µm r=0.51 (95% CI= [0.12; 0.77]) P= 0.01 and r=0.55 (95% CI= [0.20; 0.78]) P= 0.003, respectively (Figure 3:7 a & b). (Hathway et al., 2013; Mirhoseini et al., 2015; Huang et al., 2017)

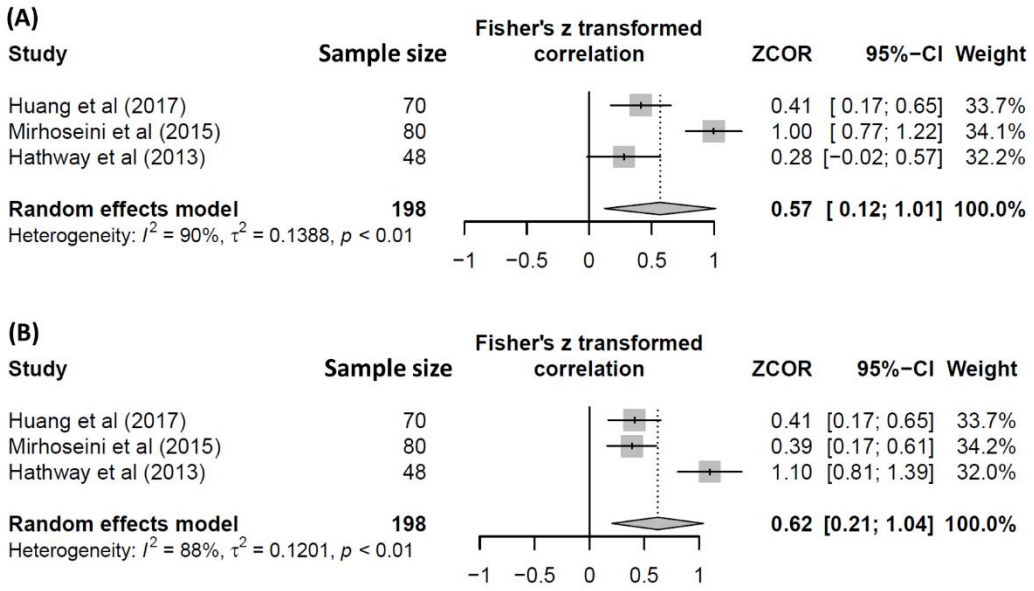

Figure 3:7 Forest plot for the studies reporting relationships between particulate matter size and airborne microbial concentration using Fisher's transformed correlation. (a) correlation with particulate matter of size ≤5 µm. (b) correlation with particulate matter of size >5 µm.

Airborne particulate matter may be indicative of the transport and deposition of a microorganism in air, and where microorganisms are released alongside other particle generating activities it is important to understand whether particle measurement is a useful proxy for microorganisms. It is evident that particulate matter of size < 5 µm are likely to be of greatest importance as they fall within the size range of bioaerosols that can remain airborne for long periods of time (between 100–1000 s) (Mingotti et al., 2020) This study result shows that there is significantly moderately positively correlation between airborne microorganisms, particle mass concentration (≤5) µg/m^3 and diameter particle concentration (≤5 and >5µm) particle/m^3 , while not significantly correlated with particle mass concentration of >5 µg.

It is hard to determine whether these relationships between microorganisms in the air and particles are directly or indirectly a result of the hospital environment. Previous studies illustrate that increased activity in hospital wards (e.g. patient bathing or wound toilet behind closed curtains) is correlated with increased concentrations of bioaerosols and particles; wards are generally full of patients, healthcare workers and visitors leading to contamination and re-contamination of the environment (Hathway et al.,

2013; La Fauci et al., 2017). Additionally, human occupancy has a strong link with indoor particle mass concentration (Licina et al., 2016). Much higher particle mass concentrations may however be associated with outdoor air pollution which would not be expected to be correlated to microorganism sources within a hospital ward. As a result, the directed acyclic graph method (Figure 3:2) suggests that this could be a factor which could be controlled for in future measurement studies. A recent study based on a simplified model experiment highlights that the movement of people may play a significant role in dispersing of aerosols of size 5–10 μm for 15 m away from the original sources in corridors and likely in rooms a building (Mingotti et al., 2020).

3.3.5 Correlation between ACC and TF level in the air

The final analysis considered the correlation between ACC and TF, and this was measured by more studies. The sample size ranged from 4-96 with a total of 305 values across the ten studies. (Jaffal et al., 1997; Augustowska and Dutkiewicz, 2006; Tekİn et al., 2013; Me´heust et al., 2013; Huang et al., 2013; Park et al., 2013; Yang et al., 2014; Fekadu and Getachewu, 2015; Sajjadi et al., 2016; Božić et al., 2019). The pooled estimated was moderately positive r=0.31 (95% CI= [0.07; 0.52]), P=0.014 (Figure 3:8).

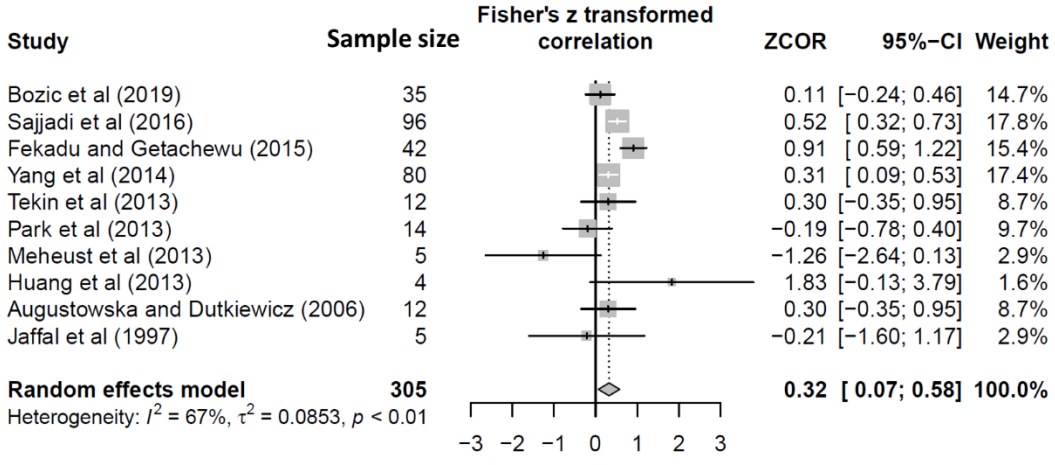

Figure 3:8 Forest plot for the studies reporting relationships between the airborne microbial concentration and airborne total fungi concentration using Fisher's transformed correlation.

Bacteria and fungi (ACC and TF) may be generated from patients and HCW activities, human shedding and from the environment that surrounds the location of sampling. The studies identified in the review show high variability in measurements between different studies in different locations which may be influenced by multiple parameters as illustrated in Table 3:1. A small number of studies show fluctuation with time in the same location (Hathway et al., 2013), and demonstrate the complexity of interactions, with microorganisms, particles and CO_2 concentrations all affected by the number of people and the activities taking place. However, while humans are considered to be the predominant source of bacteria in hospitals, most airborne fungi in NHS hospitals come from the outside environment, water tanks, or from mould permitted to contaminate damp areas (and not cleaned properly). Thus, the correlation between bacteria and fungi can be misleading if the study does not take into account the type of environment, activities and sampling intervals.

3.3.6 Limitations

For most of the analysis, data is drawn from a small number of studies, although the total numbers of samples across all the studies are larger. These studies are carried out across multiple different hospitals across 11 different countries and a wide range of different ward and clinical spaces. These countries will all have different healthcare systems, hospital design, hospital management and patient mix. It is therefore possible that the observed negative and positive correlations could be attributed to confounding variables that change the direction and strength of the relationship (Rohrer, 2018). Previous work shows that the infection status of the patients (not colonised, infected and/or colonised) and clinical care activities are both correlated with increased concentrations of airborne microorganisms (Sexton et al., 2006; Hathway et al., 2013; Dougall et al., 2019). Hence confounding variables, such as patient conditions, number of people, healthcare worker activity and indoor area under study, need to be controlled in analysis, for example, through the use of multivariate regression to estimate the effect size. As discussed above the design of the healthcare ventilation, heating and air conditioning systems will vary by country and this will further impact on the measured microbial burden as well

as the IAQ parameters. As the number of studies is small, these parameters cannot be stratified to control for this potential confounding.

This review highlights the lack of good data on relationships between microorganisms and environmental conditions from healthcare settings, with most of the knowledge on these factors derived from controlled laboratory studies. While there are numerous studies that have sampled microorganisms in hospitals, there are very few studies that are designed to be able to capture the full range of environmental, activity and microbial information. Despite the fact that there are several papers demonstrating the effect of healthcare worker presence and activity on releasing or dispersing microorganisms,(Hathway et al., 2013) these normally occur during a sampling snapshot, and there is little data on the influence of these activities on the dispersion and deposition of microorganisms over time. Few studies are able to provide evidence on causation, and therefore future research needs to investigate the combined mechanisms in real-world settings that underpin or cause IAQ parameters to influence the dispersion, survival and deposition of microorganisms. Moreover, studies also need to consider the implications of any relationships for infection control. To achieve this, cross-disciplinary collaborations between microbiologists, infection control specialists with expertise in ventilation and indoor air quality are essential to design effective studies.

3.4 Summary

This chapter systematically reviewed studies that sampled airborne microorganisms in hospital wards and presented quantitative data with one or more IAQ parameters (temperature, relative humidity, CO_2, particle mass concentration and particulate matter of size). We found that there is only a small number of studies that provide quantitative data to assess relationships between airborne microorganisms and IAQ parameters from measurements made in hospitals outside of settings with specialist ventilation (e.g., operating rooms). Overall, we can conclude the following from the meta-analysis:

- There are likely to be positive correlations between airborne bacteria and other types of microorganism, particularly fungi.
- There are positive correlations between airborne bacteria and fungi, measured as ACC, and several IAQ parameters (temperature, CO_2, particulate matter of size of ≤5 and >5μm and particle mass concentration ≤5 μg/m^3). However, the data did not demonstrate a clear correlation with relative humidity, and correlations between TF and IAQ parameters were weak.
- There is only a very small number of studies that present quantitative data while measuring the environmental and activity factors that affect the presence and quantity of airborne microorganisms.

4 Quantifying the relationship between airborne microorganisms and surface bioburden in hospital environments

4.1 Introduction

It is recognised that microorganisms in the air in a hospital can contribute to surface bioburden through their deposition. However, there is not a clear understanding of how to quantify relationships between airborne microorganisms and surface contamination and the factors that affect this. There is very little data on deposition in real environments, and even computational fluid dynamics models struggle to accurately predict it or are unable to generalise to other settings. This chapter explains the theory of particle deposition and describes how the deposition rate is influenced by other environmental parameters. Then published data is used to examine the known correlations between microorganisms in the air and on surfaces and to evaluate a predictive model of the concentration of airborne microorganisms based on the surface bioburden considering guidelines for cleanliness.

4.1.1 The theory of deposition

The movement of small aerosol particles (≤ 100μm) can be calculated by equation 4.1, which integrates the force balance on the particle in terms of drag, gravity and Brownian forces

$$\frac{\overrightarrow{dv_{\mathrm{p}}}}{dt} = F_D\,(\vec{v} - \vec{v}\mathrm{p}) + \frac{\vec{g}\,(P\mathrm{p} - P)}{Pp} + F\mathrm{B} \qquad (4.1)$$

Where, v_p is the particle velocity, v is the fluid velocity,F_D is the drag force per unit particle mass. $P\mathrm{p}$ and p is particle and air densities at a specific temperature, respectively. F_B is the Brownian forces.

Stokes's law indicates that in still air the upward drag force that resists the fall of a particle can be calculated using equation 4.2 (Fuchs, 1986), and the downward gravity force is found using equation 4.1. Once the downward and upward forces become the same, the acceleration becomes zero, and the particle reaches a constant velocity called terminal velocity. Thus, the

velocity in equation 4.4 is expressed by using equations 4.2 and 4.3 and solving them for velocity.

$$F_D = 3\pi\eta R v \qquad (4.2)$$

where F_D Is the drag force, which is known as Stokes's drag (kg.m.s^{-2}), η is the viscosity of the air at 20 °C (1.81 × 10^{-5} kg.m^{-1}.s^{-1}), R is the radius of the spherical particle and v is the particle velocity.

$$F_g = (P_p - P_f) g \frac{4}{3}\pi R^3 \qquad (4.3)$$

where F_g, is the gravity force (kg.m.s^{-2}), P_p is the mass density of the particle (kg.m^{-3}), P_f is the mass density of the fluid (1 kg.m^{-3}), g is the gravitational field strength (9.81 m.s^{-2}), and R is the radius of the spherical particle.

$$v = \frac{2}{9}\frac{(P_p - P_f)}{\eta} g R^2 \qquad (4.4)$$

Assuming those living cells are spherical particles and the mass density of a living cell is 1 kg.m^{-3} (Grover et al., 2011), Figure 4:1 shows the terminal velocity (m.s^{-1}) and the settling time for 2 meters fall with different particle diameters (1-90µm).

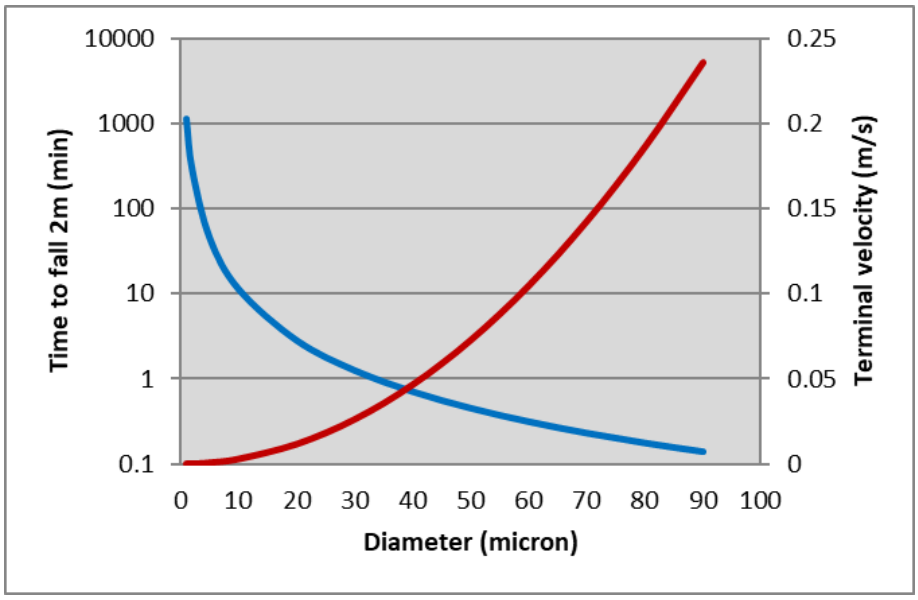

Figure 4:1 The terminal velocity(red) and settling time (blue) with different particle diameters.

In the controlled environment, the deposition velocity (m.h^{-1}) of microorganisms from the air can be estimated according to equation (4.5)

$$v = \frac{C_s}{C} \quad (4.5)$$

Where C_s is the measured deposition rate of airborne microorganisms over time on surfaces (cfu.m^{-2}.h^{-1}), and C is the concentration of airborne microorganisms (cfu.m^{-3}). The deposition rate on surface can be calculated more accurately using open petri dishes (passive sampling) rather than free hand-touched surface sampling to avoid the influence of contact plate or swab efficiency.

Many researchers use the term surface air ratio (SAR) instead of v. Additionally, other researchers are more interested in this relationship as microbial deposition loss-rate coefficient (h^{-1}) which plays an important role in modelling airborne infection risk (Miller et al., 2020). Under the steady-state conditions, the total loss rate due to deposition onto surfaces can be calculated as follows (equation 4.6):

$$\lambda_d = \lambda_{d.f} + \lambda_{d.w} + \lambda_{d.c} \quad (4.6)$$

where, λ_d is the loss rate due to deposition onto all room surfaces per hour. $\lambda_{d.f}, \lambda_{d.w}\ and\ \lambda_{d.c}$ are the loss rate due to deposition onto floor, wall and ceiling surfaces per hour. The ($\lambda_{d.f}$) can be calculated according to equation (4.7).

$$\lambda_{d.f} = \frac{C_{sf}\, A_f}{C\, V} \quad (4.7)$$

Where C_{sf} is the indoor deposited microorganisms' concentration on the floor ($cfu.m^{-2}.h^{-1}$). A_f is the internal surface area of the floor (m^2). and V is the volume of the room (m^3). However, if the room shape is cuboid, then the A/V is equal to $1/H$, where H is the height of the room. although these equations allow for estimation of deposition velocities and loss rates, they don't differentiate the behaviour of different particle sizes and the air sampling is able to size fractionate, but there are no techniques for doing this on surfaces, and the petri dish essentially collects everything.

4.1.2 Correlation between air and surface bioburden

Airborne microorganisms are assumed to eventually either be removed through ventilation or deposit onto surfaces, and that the number of deposited microorganisms over a period of time correlates to the number of airborne microorganisms. A qualitative link between air and surface microbes was found decades ago and was shown as a percentage of positive sampling (Alberti et al., 2001; Brunetti et al., 2006; Carvalho et al., 2007; Best et al., 2010). More recently, researchers found that there is a tenuous quantitative correlation of *P.aeruginosa* and total fungal load in the air and on surfaces (Huang et al., 2013; Bonnal et al., 2015). However, one study on the total fungal flora and *Aspergillus spp* shows no significant correlation between airborne fungal and surface fungal (Gheith et al., 2015).

There is currently no consensus among researchers in selecting an ideal methodology for sampling bioaerosols, and there are no unified standards for quantifying airborne microorganisms inside healthcare buildings (Nunes et al., 2005). The Microbial threshold limit for special areas such as operating rooms and areas of pharmaceutical manufacturing was recommended in Table **4**:**1**. However, regarding airborne bioburden in hospital wards, there are no clear guidelines or standards, even from the World Health Organization (WHO) (Kim et al., 2018).

The quantitative relationship between microorganisms in active and passive sampling is still unclear and needs to be supported with empirical evidence. Despite the number of studies that have sampled the hospital environment (Table 4:2), there have been few attempts to evaluate any relationships between microorganisms in the air and on surfaces. Only two studies have proposed mathematical equations to quantify this interaction in a real world environment (Omelyansky, 1940; Whyte and Eaton, 2016). Omeliansky's equation has been used in a number of studies with settle plate data to estimate the airborne concentration in hospital settings (Grieble et al., 1970; Krishna et al., 2007; Cordeiro et al., 2010; Shrestha et al., 2012; Munoz-Price et al., 2013; Hsueh et al., 2014; Jomha et al., 2014; Fekadu and Getachewu, 2015; Shimose et al., 2016; Gizaw et al., 2016). Whyte and Eaton's equation was formulated based on the data that was collected from

cleanrooms and operating rooms to be used in pharmaceutical manufacturing areas (Whyte and Eaton, 2016).

Table 4:1: Microbial threshold limit recommendation.

References	Place	Air sample cfu.m^{-3}	Settle plates cfu.90mm^{-1}.4h^{-1}	Settle plates * cfu.m^{-2}.h^{-1}	SAR	$\lambda_{d,f}$ h^{-1}
(European Committee, 2009).	EU-GMP Grade A	<1	<1			
(European Committee, 2009).	EU-GMP Grade B	10	5	196	19.65	6.6
(European Committee, 2009).	EU-GMP Grade C	100	50	1965	19.65	6.6
(European Committee, 2009).	EU-GMP Grade D	200	100	3930	19.65	6.6
(Pasquarella et al., 2000)	Operating room	35	~9	350	10	3.3

EU-GMP: European Union - Good Manufacturing Practices.

* This column is calculated from the previous column (Settle plates cfu.90mm^{-1}.4h^{-1}).

SAR is the Surface air ratio Surface (m. h^{-1}).

$\lambda_{d,f}$ is the loss rate of airborne microorganisms due to deposition onto floor surfaces (h^{-1}), which is calculated using equation (4.7), and assuming the room height is 3m.

The SAR and value of $\lambda_{d.f}$ in table 4.1 was calculated using equation (4.7).

The SAR is used to determine the concentration of deposited microorganisms on surfaces within one hour (cfu.m^{-2}.h^{-1}) from the airborne microbial concentration. Alternatively, it can also be used to find the concentration of airborne microorganisms (cfu.m^{-3}) if the surface bioburden was known. The recommended value of SAR in table 4.1 in especially hygienic area ranged from 10 to 19.65 (m.h^{-1}) and the loss rate of airborne microorganisms due to deposition onto floor surfaces ranged from 3.3 to 6.6 (h^{-1}). However, in different hospital area, the value of SAR in table 4.2 ranged from 0.26 to 112.26 (m.h^{-1}) and the loss rate of airborne microorganisms due to deposition onto floor surfaces ranged from 0.10 to

37.42 (h^{-1}). The ventilation rate and regime, the presence of people, and the varying hospital environment led to this high range of results.

Table 4:2: A summary of the studies that include air and surface sampling data in the literature.

References	Place	Ventilation type and rate ACH	no. of people presents	Other factors	Type of microorganisms	SAR	$\lambda_{d,f}$ h^{-1}
Stokes' law					Particle (1-10 µm)	0.11-11.02	0.04-3.67
(Omelyansky, 1940)	NA	NA	NA	NA	NA	11.99	4.00
(Whyte et al., 1982)	Operating room	conventionally-ventilated	NA	NA	ACC	18.00	6.00
(Hambraeus, 1988)	Operating room	conventionally-ventilated	NA	Uv off	ACC	71.90	23.97
	Operating room	conventionally-ventilated	NA	UV on	ACC	19.60	6.53
(Friberg et al., 1999)	Operating room	Displacement system (17 ACH)	NA	NA	ACC	8-15	2.67-5.00
	Operating room	conventionally-ventilated (17 AHC)	NA	NA	ACC	10-18	3.33-6.00
(Napoli et al., 2012)	Operating room (before surgery)	32 turbulent air flow	NA	NA	ACC	58.27	19.42
	Operating room (during surgery)	32 turbulent air flow	NA	NA	ACC	112.26	37.42

References	Place	Ventilation type and rate ACH	no. of people presents	Other factors	Type of microorganisms	SAR	$\lambda_{d,f}$ h^{-1}
(Wong et al., 2011)	office (microbiology department)	Natural (Closed window)	1 for 2min/15 min	NA	ACC	8.31	2.77

	office (microbiology department)	Natural (Closed window)	1 (fanning a worn laboratory coat with both hands, 10 times/15 min)	NA	ACC	17.29	5.76
	ICU (single patient-occupied)	Mechanical (12)	normal clinical activity	NA	ACC	16.71	5.57
(Whyte and Eaton, 2016)	Pharmaceutical manufacturing	20	NA	NA	ACC	5-25	1.67-8.33
(Saha et al., 2017)	Operating room (before surgery)	NA	NA		ACC	0.29 ± 0.32	0.10 ± 0.11
	Operating room (during surgery)	NA	Presence of people		ACC	0.26 ± 0.27	0.10 ± 0.09

ACC = airborne microbial concentration measured as aerobic colony count, NA= not available, SAR is the Surface air ratio Surface (m. h^{-1}), $\lambda_{d,f}$ is the loss rate of airborne microorganisms due to deposition onto floor surfaces (h^{-1}), which calculated using equation (4.7), and assuming the room hight is 3 meters.

4.1.3 Surface hygiene standards

Surface sampling is typically carried out using one of the six methods including contact plates, dipslides, swabs or sponge or gauze, filter vacuum and pumps, tape and petrifilm. The advantages and disadvantages of using them are summarised in Table 4:3 (Alberti et al., 2001; Brunetti et al., 2006; Sexton et al., 2006; Carvalho et al., 2007; Best et al., 2010; Huang et al., 2013; Creamer et al., 2014; Gheith et al., 2015; Bonnal et al., 2015; Shimose et al., 2016; Kim et al., 2016a; Killingley et al., 2016; Smith et al., 2018a). According to a previous review that considered 33 studies, all of which performed surface sampling in hospitals, the percentage of methods used in these studies was 53% for swab, 24% for contact plates, 9% for sponges, 6% for dipslides, 5% for gauze, and 3% for Petri-films (Rawlinson et al., 2019).

Table 4:3 A summary of the advantages and disadvantages of surface sampling.

Surface sampling advantages	Contact plates	Dipslides	Swabs, Sponge or Gauze	Filter vacuum and pumps	Tape	Petrifilm
Easy to use	✔	✔	✔	✔	✔	✔
Quantitative purposes	✔	Semi-quantitative	✔	✔	Only direct observation	✔
No need additional transfer stages	✔	✔	✔	Further transfer and culture stage need it to find level of contamination	✔	✔
Can get information on all species	Only one species	Two species	✔	✔	Only one species	Only one species
Cheap	Expensive than swabs	Expensive than swabs	✔	Expensive than swabs	Expensive than swabs	Expensive than swabs
Able to sample range of surfaces including non-flat	Relatively flat only (ex. hospital high touch)	Relatively flat and uneven flat (ex. hospital high touch)	✔	✔	Relatively flat and uneven flat	✔

* A flat surface indicates a plain and even surface with no curves or ridges

Several studies have used air and surface microbial sampling to evaluate the concentration of microorganisms present in the hospital environment.

There is evidence that the environment contributes to infection transmission. However, the specific relationship between airborne pathogen load, surface contamination and HAIs remain unknown (Smith et al., 2018a). The staff activities and presence of people in the hospital are also major factors in generating and releasing microorganisms into the air and hence should be considered when reporting real-world results (Shiomori et al., 2002; Moore et al., 2002; Cavallo et al., 2013; Yang et al., 2014).

Previous studies have used the information from environmental sampling to make hygiene recommendations for bioburden levels in environments such as food preparation and recreational waters (Dancer, 2004; Dancer, 2014). These and others led to the proposal of two standards for surface level cleanliness in hospitals in 2004 (Dancer, 2004). The first standard specified is an aerobic colony count (ACC) <5 cfu/cm^2 for hand-touch sites, and the second, also for hand-touch sites, is <1cfu/cm^2 for recognised hospital pathogens such as MRSA or VRE. These two standards were found to be related in that higher levels of aerobic colonies on hand-touch sites are more likely to be associated with the presence of a pathogen such as MRSA (Dancer et al., 2008). A more recent paper compared and contrasted 2.5 cfu/cm^2 and 5 cfu/cm^2 in hospitals and found that the difference between pass and fail proportions were arbitrary (Griffith et al., 2007). This was repeated in other studies and yielded similar results (Meakin et al., 2012). It has been suggested that 5 cfu/cm^2 might better reflect HAISs risk for general wards and 2.5 cfu/cm^2 for critical care units (White et al., 2008; Bogusz et al., 2013); Measurements from these and other studies provide a range of tangible values that can be modelled against the infection risk for patients over time (Dancer, 2014).

The survival rate of microorganisms on hospital fomites is also dependent on many factors, for example, the type of the microorganism, the time considered for sampling, hand contact and the residue of disinfectant used during cleaning. It is significantly important to identify potential risks of infection in a healthcare environment to adopt reasonable measures and to

minimise the probability of an infection. One of the main factors that contribute to a sound assessment is knowing the survival rate of airborne bioaerosols and surface deposited microorganisms. A study that considers the environmental contamination with Liverpool epidemic *P. aeruginosa* strains in hospital wards and out-patient clinics claims that there were no persistent reservoirs detected. The study concludes that patients may be getting the infection from short-lived contamination within a short range of them (Panagea et al., 2005). A recent study in an intensive care unit (ICU) concluded that passive air sampling provided more reliable data than active air sampling when modelled against the surface benchmark (Smith et al., 2018a).

4.2 Analysis methodology

4.2.1 Evaluation of existing mathematical equations to quantify the relationship between air and surface bioburden.

To assess the quantitative relationship between air and surface microbial load, two mathematical equations were identified that have been proposed to relate the airborne concentration with surface deposition rate over time (See table 11).

Table 4:4 Quantitative models to relate air and surface bioburden.

Author	Equation	Parameters
Omeliansky, 1940	$y = 12x$ (4.8)	y = C_s= Deposition rate of airborne microorganisms on Surfaces (cfu.m^{-2} h^{-1})
Whyte and Eaton, 2016	$y = 58\ x^{0.657}$ (4.9)	x=C= microorganisms load of indoor air (cfu.m^{-3})

These equations are first evaluated against data from Wong et al. (2011), which includes descriptive hospital data for passive sampling and active sampling and the time intervals over which the samples were taken. They include a set of 48 ACC readings which are represented by the mean values of active and passive sampling results over four hours /day, for 16 days

under three scenarios: (a) in a non-clinical room with light occupant activity, (b) in a non-clinical room with regular activity (one person fanning a worn laboratory coat with both hands, ten times, every 15 min), and (c) a patient-occupied ICU with regular healthcare worker activity.

4.2.2 Multilinear regression model considering HCW activity.

A multilinear regression model was then constructed to account for the effect of human activity on the airborne and surface bioburden. Since the data in Wong et al. was not normally distributed, Spearman's Rho was applied to evaluate correlation. For both a simple and multilinear model, a general linear model was constructed to provide coefficients, adjusted R-square and standard error of the estimate values.

$$y = 0.25 + 1.17 \times 10^{-3}x + 0.16\,a \qquad (4.10),$$

where, x and y are as per Table 4:4 and a relates to an activity level:1= low and 2= regular.

4.2.3 Receiver operating characteristics curves (ROC) approach

The passive sampling and calculated surface results for ACC were recorded as a binary "pass" or "fail" according to the benchmark of 5 cfu/cm^2 on surfaces defined in previous work (Dancer, 2014; Smith et al., 2018a). Using the receiver operating characteristics curves (ROC) and area under the curve methods, the ability of the Omeliansky and Whyte & Eaton equations and the simple and multilinear regression models to predict surface contamination for a binary classifier (no-risk= 0 and risk =1) are evaluated. SPSS version 25 was used to perform these analyses. Significance levels were considered as 0.05.

4.3 Result and discussion

4.3.1 External validation of existing equation

The predicted rate of surface contamination from the data in Wong et al., 2011 was made using both the Omeliansky, Whyte & Eaton equations and a simple linear model equations shown in figure 4.2.

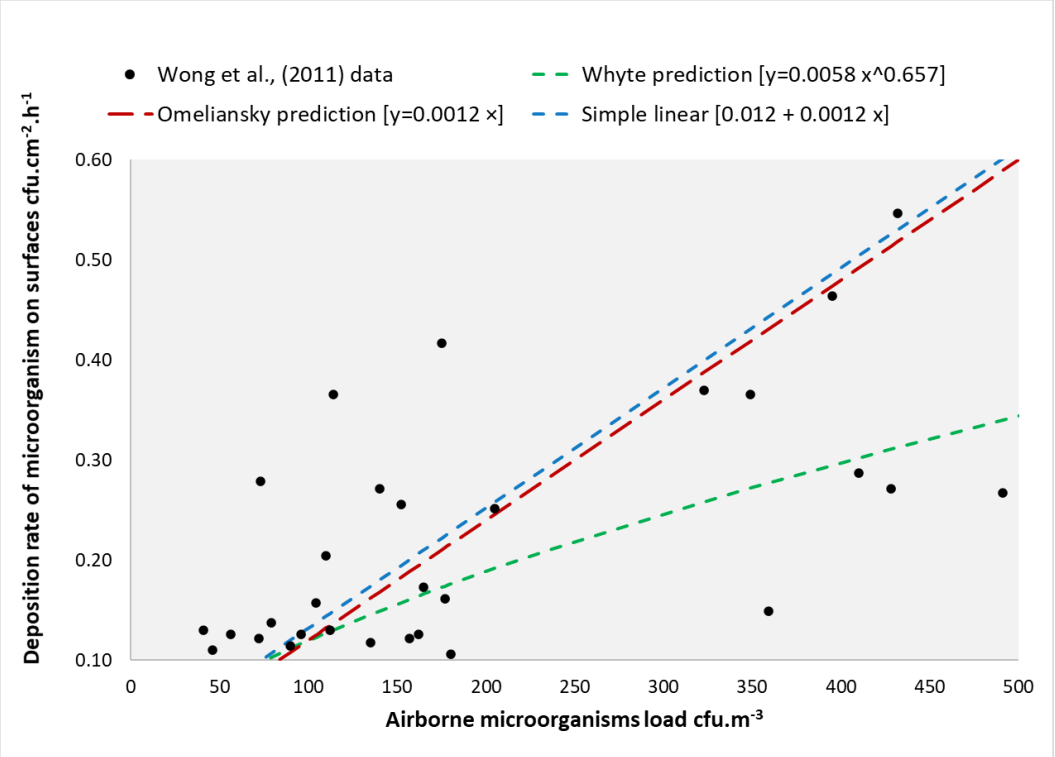

Figure 4:2 : The data together with the Omeliansky and Whyte & Eaton equations and the simple linear model

4.3.2 Multilinear regression model considering HCW activity.

Visual inspection of variable correlations of parameters (airborne microorganism level, deposition rate of microorganisms and human activity level) and nonparametric correlation calculations suggest there is a significant relationship between the deposition rate of microorganisms and activity ($r=0.39$, $P<0.006$) while there is no significant difference between airborne microorganism load and activity ($r=0.15$, $P<0.30$). Thus, the multilinear regression model was developed to consider the effects of human activity with the airborne microorganism load (Figure 4:3). The activity of the first scenario is represented as “1” (light activity), and the second and third scenarios are represented as “2” (regular activity). A multilinear regression model $r=0.70$ (95% CI= [0.52; 0.82]), $P<0.001$ is significantly ($P=0.018$) better than the simple linear model $r=0.65$ (95% CI = [0.44 - 0.79]), $P<0.001$ (Table 4:5)

Table 4:5 Linear correlations between the predictions from Omeliansky, Whyte and Eaton and regression equations with and without activities against the measured data from Wong et al., 2011.

		Wong et al. data		Predictions		Our linear regression model	
Equation		Air (178 ± 159) cfu/m3	Surface (0.22 ± 0.29)	Omeliansky (0.21 ± 0.19)	Whyte & Eaton (0.16 ± 0.10)	Simple (0.22 ± 0.15)	multiple (0.22 ± 0.17)
			cfu/cm2 /h				
Air		1					
Surface	r	0.790*	1				
Omeliansky $y = 1.20 \times 10^{-3} x$	r	1.000*	0.790*	1			
Whyte and Eaton $y = 5.80 \times 10^{-3} x^{0.657}$	r	1.000*	0.790*	1.000*	1		
Simple $y = 0.012 + 1.17 \times 10^{-3} x$	r	1.000*	0.790*	1.000*	1.000*	1	
Multiple $y = 0.25 + 1.17 \times 10^{-3}$ x $+ 0.16\, a$	r	0.898*	0.816*	0.898*	0.898*	0.898*	1

Y = rate of surface contamination (cfu/cm2/h), x = microorganisms load of indoor air (CFU/m3), a= level of activities (1 & 2)

* = Significant at 0.01 level

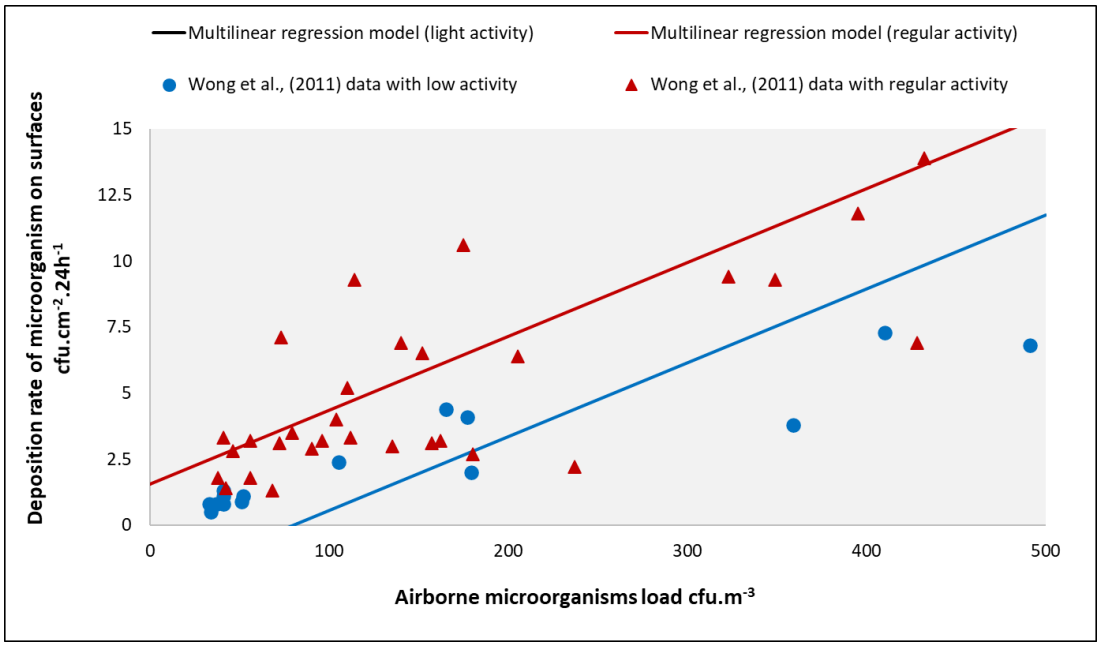

Figure 4:3 Predicted versus actual microorganism load deposition in cm^2 per hour for the Omeliansky and Whyte &Eaton models.

4.3.3 Receiver operating characteristics curves (ROC) approach

The receiver operating characteristics (ROC) curve is a useful statistics technique for organizing binary classifiers and illustrating their performance in analysing the strength/predictive power of a classifier (Fawcett, 2006). It plots the true positive rate of data prediction (sensitivity) in y axis against the false positive rate (1-specificity) in X-axis at all classification thresholds to determine the optimal probability threshold for a classification model.

$$TPR = \frac{TP}{TP + FN} \tag{4.11}$$

$$FPR = \frac{FP}{TN + FP} \tag{4.11}$$

Where, TPR is true positive rate, TP is the number of true positives, FN is the number of false negatives, FPR is the false positive rate, FP is the number of false negative and TN is the number of true negative.

The model that has a curve closer to the top-left corner of the ROC indicates that it has a better performance. Otherwise, a model that produces a curve further from the top-left corner towards the diagonal 45° line indicates a worse performance.

Based on analysis using ROC, (Figure 4:4) shows the results for predictions using the multilinear and simple regression models and Omeliansky, Whyte & Eaton predictions yield a c-statistic of 0.921 (95% CI= [0.82; 1,00]), 0.876 (95% CI=[0.76; 0.99]), 0.875 (95% CI= [0.76; 0.99]), 0.869 (95% CI= [0.75; 0.99]), respectively.

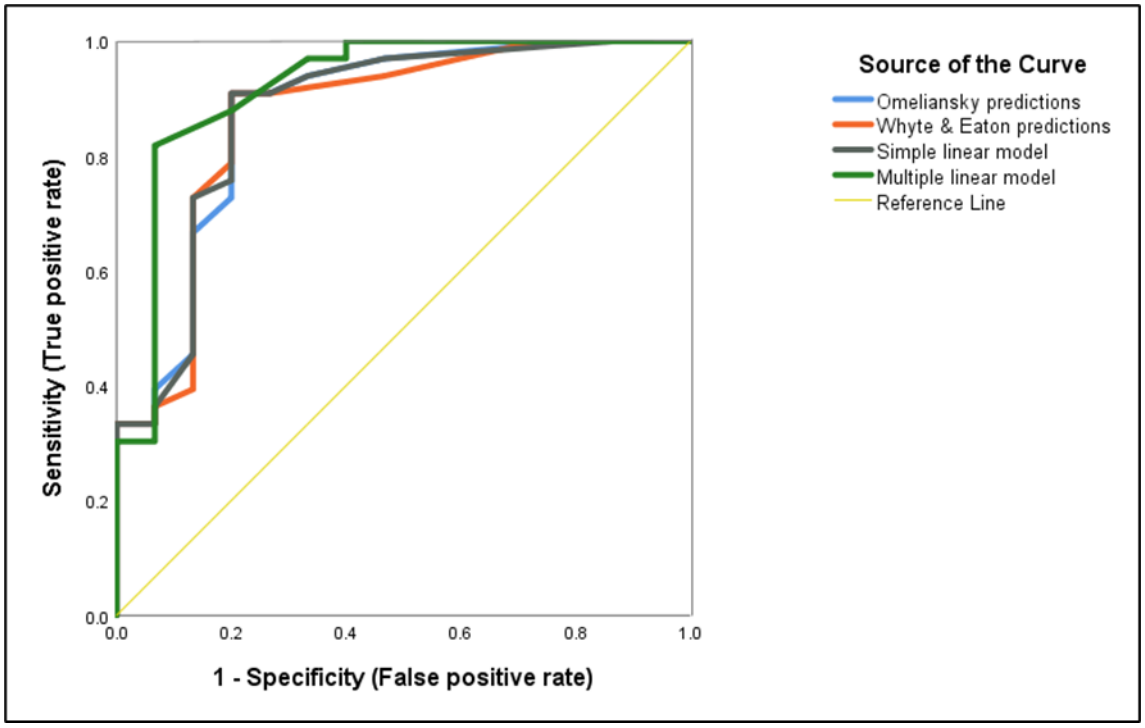

Figure 4:4 Receiver operating characteristics curves showing the discriminative value of all models.

Predictions using Omeliansky's equation and Whyte and Eaton's equation were both significantly correlated with microorganisms load measured by Wong et al., 2011, suggesting that they are both broadly representative of behaviour in a real environment.

Further analysis suggested that activity had a significant correlation with the deposition rate of microorganisms but not the load of microorganisms in the air. Therefore, a multilinear regression model (deposition rate model) was applied. This showed a significantly better prediction than the simple linear model. In addition, the multilinear regression model is considered a better model for assessing the risk of exceeding a benchmark value (logistic regression model). This is because the C-statistic was 0.921 (95% CI 0.826 to 1,000) for the area under the curve, and the confidence intervals were ≥

0.8. The other three models are a fair model (confidence intervals was < 0.8) (Hosmer and Lemeshow, 2003).

4.3.4 Airborne microorganism concentrations in hospital wards

The benchmark value of microorganisms on a surface is suggested to be less than 5 cfu.cm^{-2} to minimise the infection risk. Cleaning in hospital wards usually occurs at least every 24 hours; thus, the deposited microorganisms on surfaces within 1 hour (y) should not exceed 0.208 cfu.cm^{-2}.h^{-1} (5 cfu.cm^{-2} /24 h). Then, our multiple linear regression model in equation (4.10) can be used to find the average concentration of airborne microorganisms in the hospital wards equation (4.11).

$$x = \frac{y + 0.25 - (0.16 \times a)}{1.17 \times 10^{-3}} \qquad (4.11)$$

Where, a= regular level of activity (2). According to the deposition rate model, the average airborne microorganism concentration should be less than 118 cfu/m^3 in a hospital room with regular activity to avoid exceeding the benchmark risk level of 5 cfu/cm^2 on surfaces after 24 hours. This assumes that cleaning of surfaces is carried out once every 24 hours, that 100% of surface microorganisms survive and that there are no other sources of microbial contamination. This could be considered for certain hardy microorganisms, such as those that form spores and for surfaces that are infrequently touched. For some microorganisms, there will be decay over time, and for some surfaces there will be contamination through actions such as hand touching or spillages on surfaces.

4.4 Summary

This chapter discusses the relationship between airborne microorganisms and surface bioburden in hospital environments. It presents the theory behind deposition of airborne particles and explains the mathematical equations that can be used to measure the deposition rate of microorganisms on surfaces. It also shows that the concentration of airborne microorganisms can be calculated using the information obtained from passive sampling methods (Open Petri-dishes).

Additionally, it shows the following observations:

- The multilinear regression model with considerations to the level of activity is significantly better than the simple linear model and the two mathematical equations (Omliansky and Whyte and Eaton) to predict the concentration of surface bioburden.
- There is currently no clear recommendation for the accepted level of airborne microorganisms. However, our multi-regression model and the accepted benchmark value, 118 $cfu.m^{-3}$ is recommended to be the accepted level of airborne microorganisms in a hospital ward.
- The field needs intensive data on measuring the airborne concentration (using both active and passive samplers), the surface bioburden, and the activities of healthcare workers. The data should not represent a snapshot but rather provides information on a sufficient period of time (8 hours to 24 hours). This data can also either confirm or disprove the effect of the activities of healthcare workers and investigate the other factors that influence the quantitative relationship between air and surface bioburden.

5 Measuring airborne microorganisms and surface bioburden in a hospital environment

5.1 Introduction

This chapter uses experimental approaches to measure the transient relationships between air and surface bioburden in a hospital ward taking into account the presence of healthcare workers. It considers the fluctuations in microbial contamination over the day and provides suggestions for the accepted level of airborne microorganisms in hospital wards based on the deposition rate of airborne micrograms on surfaces over time and the cleanliness threshold. The aim of this chapter can be divided into four main points:

a) to investigate the quantitative relationships between both airborne aerobic colony count (ACC) and *Staphylococcus spp*, hospital environment including indoor air quality parameters, and human presence and healthcare worker activities.

b) to investigate the quantitative relationship between the deposition rate of airborne microorganisms (ACC and *Staphylococcus spp*) and the hospital environment.

c) to provide a mathematical model that relates airborne microorganisms' concentration and surface load, which could be used to calculate airborne microorganisms as cfu/m^3 when using passive sampling method (open Petri dishes); and

d) to quantify the contribution of airborne microorganism load on the surface load in the healthcare environment.

5.2 Sampling methodology

The majority of the study was performed in a 4-bed adult room, with some additional samples taken in a single-bed and a 10-bed adult room in the respiratory ward at St James's University Hospital, Leeds. The study was carried out during February (17^{th} and 21^{st}) and March (3^{rd} and 10^{th}) 2020 (Figure 1). The ward is naturally ventilated via opening windows. Bed occupancy ranges from 75 – 100%, each patient is nursed on a 1:1 basis.

Domestic and near-patient sites are cleaned once daily around 12:00 midday.

Microbiological samples in the respiratory ward at St James's hospital (in England) were taken to measure the bioburden of the air and surface in the patients' rooms while simultaneously recording the environmental conditions, the occupancy levels and the activities (Figure 5:1). The active air sampling process followed the same approach used by Hathway et al., (2013), which studied the correlation between airborne concentration and particle size. Our study includes passive sampling and surface sampling in addition to active sampling to find the deposition rate of airborne microorganisms. The sampling procedure followed a similar approach to that of Smith et al., (2018a) with the exception that our work considers producing time-series data. This can provide a better understanding of the quantitative relationships between air and surface bioburden during a full working day.

Additional data was planned to be collected through further sampling in the same ward during different seasons and in a different hospital (in Scotland) to establish whether the results will be consistent regardless of the time of the year and the geographical location. However, from March 2020 these wards have housed COVID-19 patients, which prevented the further sampling from taking place.

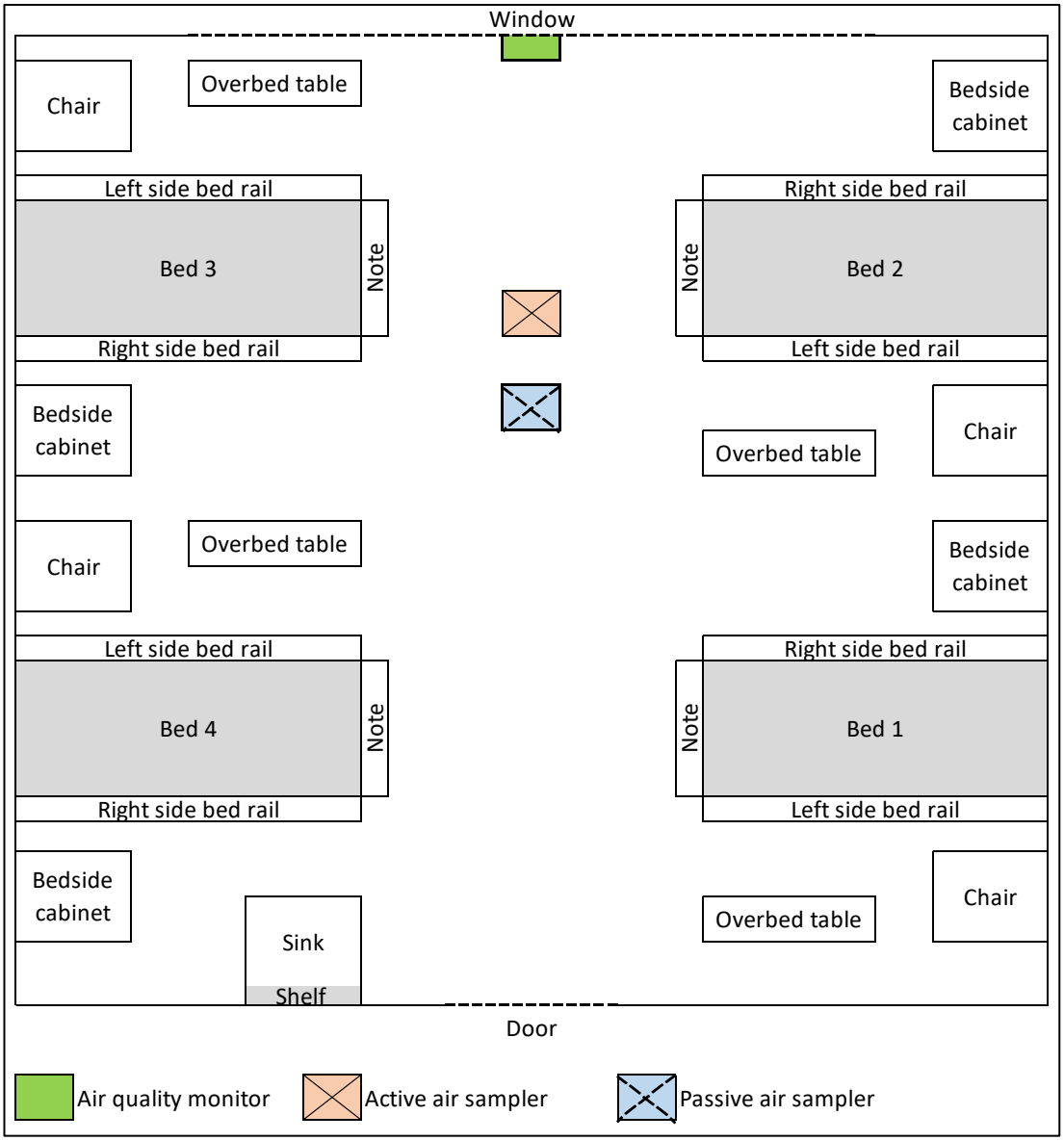

Figure 5:1 Layout of study bay indicating sampling locations.

5.2.1 Study days

Four study days were selected for air (active and passive) and surface sampling and observation of the number of healthcare workers (HCW) and their activities. Active and passive air sampling was conducted and HCW numbers and activities were recorded between 8:30-16:00 in a 4-bed ward; surface screening of five hand-touch sites around each occupied bed was carried out in the morning (9:00) and evening (16:00) in the 4-bed ward. Surface screening was also carried out in a single-bed room and a ten-bed ward, however air samples and activities were not recorded.

5.2.2 Air quality monitor

Air quality measurements of CO_2, temperature, relative humidity and particulate matter (PM 2.5 and PM 10) were performed in the four-bed ward throughout using a portable AirVisual node quality monitor (Myecohub Ltd, Ireland). The device was confirmed with medical physics, and estates teams and PAT tested prior to deployment, and it was placed on the window board (Figure 5:2). This location was chosen so that the node is situated near an electrical socket, away from other hospital equipment in locations that do not interfere with everyday care duties. For safety and convenience, it was placed as far away from patients and visitors as possible. One disadvantage is that the location close to the window may result in some values being influenced by the window. The air quality parameters were measured using a similar approach to Fifield et al., (2018). The data was recorded every 12 minutes and was then downloaded at the end of the measuring period.

5.2.3 Microbial sampling

Bacteria are more widely studied (53%) than fungi (42%) or viruses (5%) in hospital settings (Chapter 2,3,3). For bacteria, airborne microbial concentration measured as aerobic colony count (ACC) and *Staphylococcus spp* were the most interesting target for researchers as it is related to the standard hygiene recommendations (Dancer, 2004; Dancer, 2014). For ACC, it can be using tryptone soya agar (TSA) as the common media for growth of all types of bacteria and using mannitol salt agar (MSA) for *Staphylococcus spp.*

5.2.3.1 Preparing Petri dishes plates

Tryptone Soya Agar (TSA) Oxoid Ltd, UK and Mannitol salt agar (MSA) Oxoid Ltd, UK were used to prepare Petri dishes plates 90mm. An amount of 40g of TSA or 111g of MSA was added to one litre in the Masterclave 09 (Don Whitley Scientific) which was used to prepare the sterile agar broth. The agar mixtures were stirred for 15 minutes, and then they were heated to 121°C for 15 minutes. The agar was then cooled and left at a constant temperature of 45°C. An automated pourer stacker (Don Whitley Scientific) was used to pour the agar broth into sterile Petri dishes (37 ml/ Ø 90mm plate); this volume was recommended by (Mcdonagh et al., 2013).

5.2.3.2 Room Air sampling

Air was sampled onto (TSA) (Oxoid, UK) to determine ACC or onto (MSA) (Oxoid, UK) for *Staphylococcus*. Active air sampling was placed between beds 2 and 3 at 1.5 m height (Figure 5:1). This presents the best place for avoiding any high changes in airflow and/or local bias in the releases bacteria caused by windows, doors, sinks, movement of patients, visitors, or HCWs. Active air sampling was performed for 5 minutes every 15 minutes for each type of ACC and *Staphylococcus spp.* (e.g. 9:00-9:05 for ACC, 9:07-9:12 for *Staphylococcus*, then 9:15-9:22 for ACC so on) using a single-stage impactor Microbio MB2 bioaerosol sampler (Fred Parrett, UK) at an airflow of 100l/min (Hathway et al., 2013). This sampler is easy to set up, portable with batteries, and has been used by previous researchers in a hospital setting (Hathway, 2011; Hathway et al., 2013). Appendix B - 400 Hole Count Correction Table was used to apply positive hole correction for the sampler (Cantium Scientific Limited, 2018) to correct for potential over-counting under higher bioaerosol concentrations. For the best results and to avoid the overestimates and underestimates that may be caused by applying the hole correction factor it is recommended that the airborne bacterial load should be between (50-200 cfu per Petri dish). Therefore, a pilot study was conducted in the same four-bed wards to determine the best sampling time.

For passive air sampling, two TSA and two MSA agar settle plates (90 mm) were placed on a 1 m high trolley that is 0.75 m away from the active sampler and at the centre between the four beds (Figure 5:2). As the aim of the sampling was to record the time series throughout the day, it was not possible to take replicate samples at the same time. Passive sampling was performed every 30 minutes for ACC and every 1 hour for *Staphylococcus*, for the first day and then every 30 minutes for both for the rest of three study days. A pilot study was performed in the same four-bed wards to determine whether the 30 or 60 minutes were enough to catch a certain number of bacterial colonies.

Figure 5:2 Microbio MB2 bioaerosol sampler and open Petri dishes in St. James's hospital.

5.2.3.3 Surface screening

The surface screening was performed using 55 mm contact plates with TSA (Oxoid, UK) for ACC and MSA (Oxoid, UK) for *Staphylococcus spp* (Figure 5:3). Five high hand-touch surfaces (bedside cabinet, overbed table, patient's note, right side bed rail and left side bed rail) were selected for screening in the morning (9:00) and in the evening (16:00) to allow comparison with previous work (Dancer, 2004; Dancer, 2009; Bogusz et al., 2013). The contact plates were placed at each surface for 10s with slight pressure and then removed, covered, and sealed (Smith et al., 2018a).

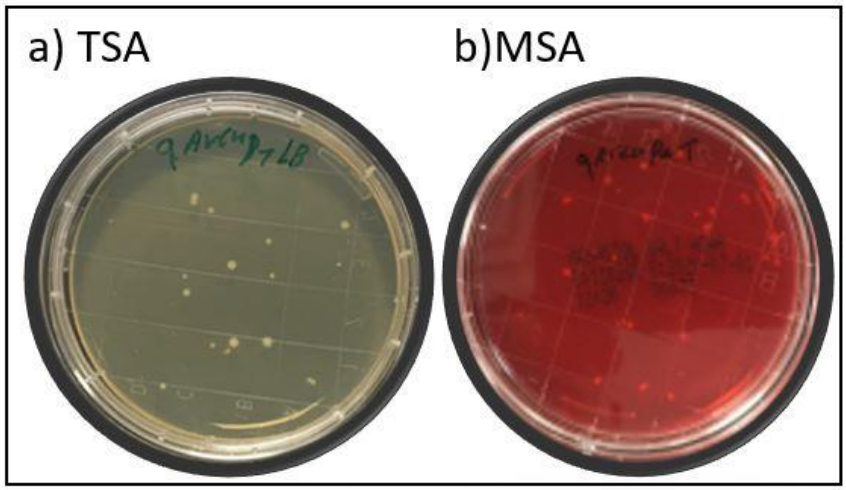

Figure 5:3: Contact plate (Ø 55 mm) with a) Tryptone Soya Agar (TSA) and b) Mannitol Salt Agar (MSA)

To quantify the contribution of airborne microorganism load on the surface load in the healthcare environment we compared the accumulated bioburden load on surfaces measured through the passive air sampling with the surface screening using contact plates. Comparison was made with sampling, which was performed around 4 pm, where the cleaning has occurred at around 12 pm.

5.2.4 Occupancy level and activities recording

Healthcare workers (HCWs), patients and visitors were recorded continuously from 8:30-16:00 for all study days. Observed activities included: talking, giving a meal, feeding a patient, giving a medicine, nurses' observation, doctor's observation, bedmaking, washing of patients (this was assumed when curtains were closed), physiotherapy, using a ventilator, cleaning, when windows/doors were opened/closed and when curtains were opened/close, and presence of visitors in the bay. For quantitative comparisons of the presence and the activity levels of patients, visitors and HCWs, a metric was created as the number of people performing an activity within 15 minutes, multiplied by the actual duration time they were in the. For example, if three HCWs were present in a ward, two of them stayed for 5 minutes, and the other stayed for 10 minutes (Within the 15 minutes timeframe), the presence and activity level metric would be 20.

5.2.5 Analysis and statistics

Air quality parameters were recorded every 12 minutes. Since our microbial data corresponds to a 15 minutes timeframe, the air quality parameters were presented to reflect the appropriate data every 15 minutes. When two different readings fall within the same 15 minutes frame, their average value is recorded instead.

For active sampling, the colonies on TSA or MSA Petri dishes measured at 100l/min for 5 minutes were counted (cfu per0.5 m^{-3}), then the correction factor was applied, and the number of corrected colonies was multiplied by 2 to represent the result as $cfu.m^{-3}$. For passive sampling, the colonies collected in TSA or MSA opened 90mm diameter Petri dishes (the surface area is 63.62 cm^2) for 30 minutes were counted (cfu/plate/30 minutes). Thenumber was then normalised by multiplying it by a value of 157.23 (10000 cm^2 / 63.62 cm^2), then by 2 to present the deposition rate as $cfu.m^{-2}.h^{-1}$.

To evaluate the correlation between the airborne microbial concentration and *Staphylococcus spp.,* the average level of ACC was calculated before and after *Staphylococcus spp.* reading time, and the mean value was used for comparison with *Staphylococcus spp*. For example, if ACC was sampled at time 9:00 and 9:15 and *Staphylococcus spp.* was sampled at 9:07, then the mean value of ACC concentration before and after 9:07 will give a more reliable value as the fluctuation is high.

5.3 Results and discussion

5.3.1 Correlation between air and hospital environment

The descriptive statistics of airborne microorganisms' concentrations (ACC and *Staphylococcus spp)* dishes in the four-bed ward are shown in Table 5:1. The average concentration of airborne microbial concentration measured for ACC was 196 ± 103. This level of ACC in the air is of a similar order of magnitudes and only slightly less than that found in a previous study which reports 241± 152 ($cfu.m^{-3}$), for experiments in a similar four-bed bay in the same respiratory ward during August 2007 (Hathway et al., 2013). The lower amount could be due to multiple reasons including the patients and

activities on the ward at the time, the ventilation conditions, or the standards of hygiene within the ward in addition to the different seasons in which the experiments were conducted (Feb-Mar as opposed to Aug). This study was carried out immediately pre-covid and this could have influenced activities on the ward.

Table 5:1 Descriptive statistics of air sampling in the four-bed ward.

	Microorganisms load in air (cfu.m^{-3}) Mean ± SD (Min-Max), Sample size	
	ACC	*Staphylococcus spp.*
Day 1 (17 Feb 2020)	194±111 (76-554), n=32	187±106 (72-430), n=32
Day2 (21 Feb 2020)	179±91 (70-508), n=32	129±51 (74-258), n=32
Day3 (3 Mar 2020)	179±117 (70-570), n=32	118±72 (47-319), n=32
Day4 (10 Mar 2020)	230±86 (114-580), n=32	198±86 (55-472), n=32
Average	196±103 (70-580), n=128	158±86 (47-472), n=128

ACC: airborne microbial concentration measured as aerobic colony count

5.3.1.1 The relationship between airborne total microbial concentration (ACC) and *Staphylococcus spp. spp.* concentration

The concentration of ACC was plotted against the concentration of airborne *Staphylococcus spp* (Figure 5:4). The concentrations of ACC and *Staphylococcus spp* in the air are similar in the order of magnitude. This shows that *Staphylococcus spp* presents (86% ± 35%) of ACC. Some sampling points were found where the *Staphylococcus spp* load is higher than the ACC load. This could be explained by the fluctuation in airborne microorganisms, which is relatively high within 15 minutes intervals. There is a positive strong significant relationship between ACC and *Staphylococcus spp.* r=66 (0.55-.075), *p*=<0.001, n=128.

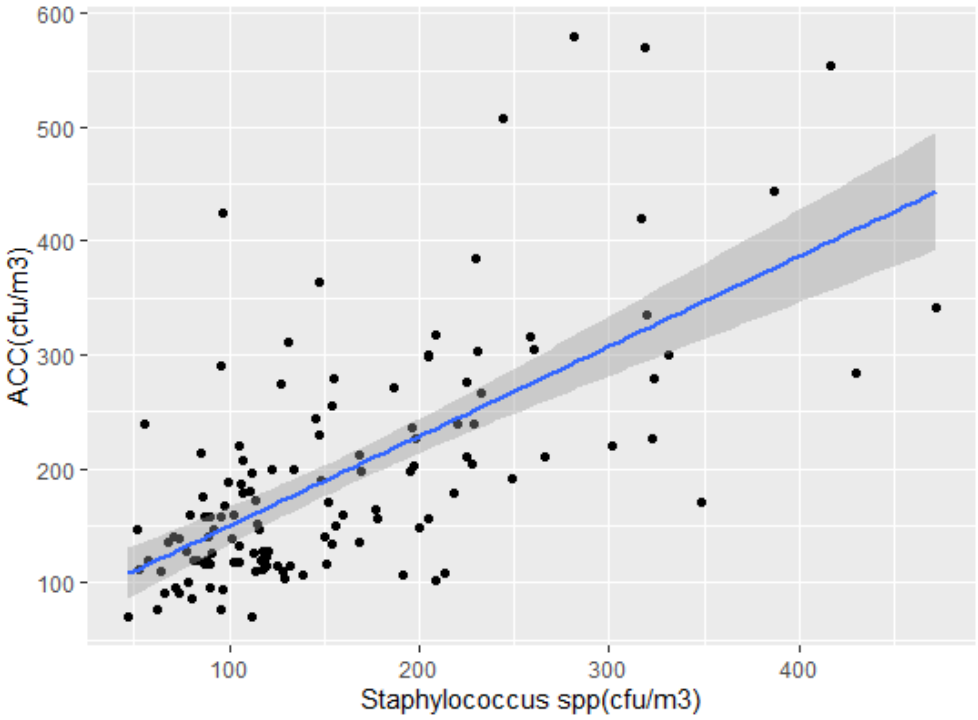

Figure 5:4 The relationship between airborne microbial concentration measured as aerobic colony count (ACC) and *Staphylococcus spp*.

The concentration of ACC can be calculated by using a simple linear regression model (5.1).

$$y = 0.7896x + 70.777 \quad (5.1),$$

Where, y is airborne microbial concentration measured as aerobic colony count (ACC) (cfu.m^{-3}) and x is airborne *Staphylococcus spp*. (cfu.m^{-3}).

The average ratio of the concentration of airborne *Staphylococcus spp*. to the concentration of ACC is 81% (158±86 cfu.m^{-3}), while the previous study reported a ratio of 56% (136 ±68 cfu.m^{-3}) (Hathway et al., 2013).

5.3.1.2 The relationship between airborne microbial concentration and indoor air quality parameters

Temperatures recorded within the four days had an average of 19±2°C with a range between 15°C to 23°C (Table 5:2). Figure 5:5 shows the scatterplot of the concentration of ACC and *Staphylococcus spp*. in the air with IAQ parameters. The temperature was not significantly correlated neither with the concentration of ACC (rho=0.08, *P*=0.34) nor with *Staphylococcus spp*. in

the air (rho=0.11, *P*=0.21). This finding is different to our meta-analysis study (Chapter 3) which found a weekly positive correlation between airborne ACC load and temperature (r=0.25 [95% CI=0.06-0.42], *P*=0.01) with a sample size ranging from 12-80 with a total of 300 values over six studies (Hiwar et al., 2021).

Table 5:2 Descriptive statistics of indoor air quality parameters.

	Day 1 (17 Feb 2020)	Day2 (21 Feb 2020)	Day3 (3 Mar 2020)	Day4 (10 Mar 2020)	Average
	Mean ± SD (Min-Max), sample size				
Temperature (°C)	18±2 (15-22), n=32	19±2 (15-21), n=32	20±1 (19-23), n=32	20±1 (18-21), n=32	19±2 (15-23), n=128
Humidity (RH)	48±5 (39-59), n=32	51±3 (45-57), n=32	43±3 (38-47), n=32	60±3 (55-66), n=32	50±7 (38-66), n=128
CO_2 (ppm)	690±147 (488-1168), n=32	524±44 (433-579), n=32	655±68 (546-793), n=32	707±69 (572-847), n=32	644±115 (433-1168), n=128
PM2.5 (ug/m^3)	18±17 (4-75), n=32	29±45 (3-151), n=32	14±31 (1-141), n=32	16±25 (2-111), n=32	19±32 (1-151), n=128
PM10 (ug/m^3)	24±23 (5-98), n=32	38±63 (3-213), n=32	20±46 (1-212), n=32	21±35 (2-150), n=32	26±44 (1-213), n=128

There was a little fluctuation of airborne microorganism from 8 am till 1 pm while it was steady after that during all days Figure 5:6. This level of temperature falls within the recommendations level 18-28 °C in ward areas for UK hospitals (Department of Health, 2007). The relative humidity recorded within the four days was 50% ± 7% (38% - 66%), as shown in Table 5:2 and Figure 5:7. This result falls within the recommended range of 30-60% relative humidity that is usually recommended in clinical areas (Sheerin et al., 2020). In our study, the concentration of ACC and *Staphylococcus spp.* in the air were significantly correlated with relative humidity (rho=0.33 [95%CI=0.16-0.48], *P*=0.007, n=128), (rho=0.39 [95%CI=0.22-0.53], *P*<0.001, n=128), respectively Figure 5:5. However, the meta-analysis in Chapter 3 (Hiwar et al., 2021) shows that the relative humidity was not significantly correlated with ACC in other studies.

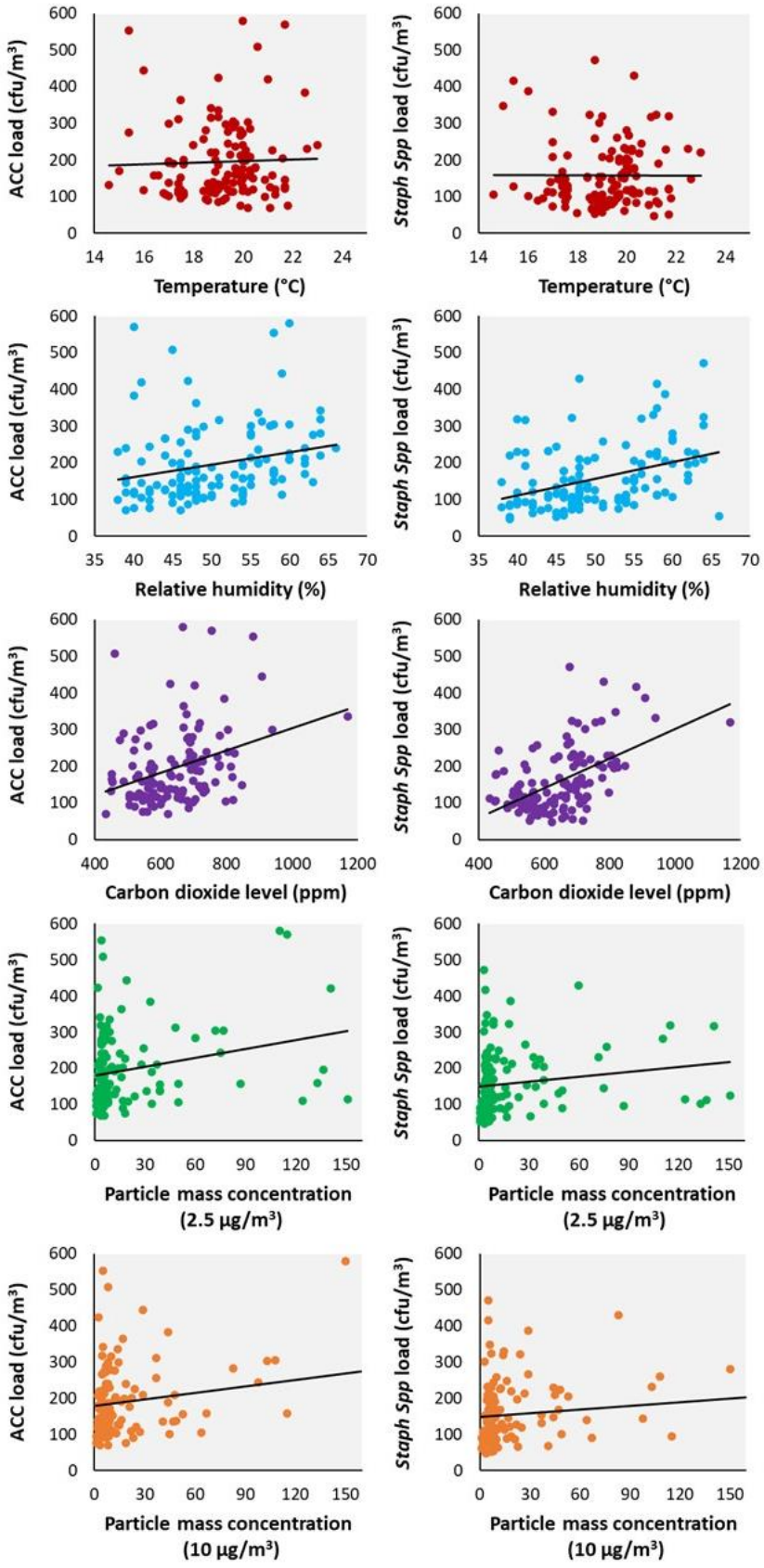

Figure 5:5 The relationship between the concentration of (ACC) and S*taphylococcus spp*. in the air (*Staph spp*) with temperature (°C), relative humidity (%), Carbon dioxide level (ppm), particle mass concentration (PM 2.5 µg/m^3) and (PM10 µg/m^3).

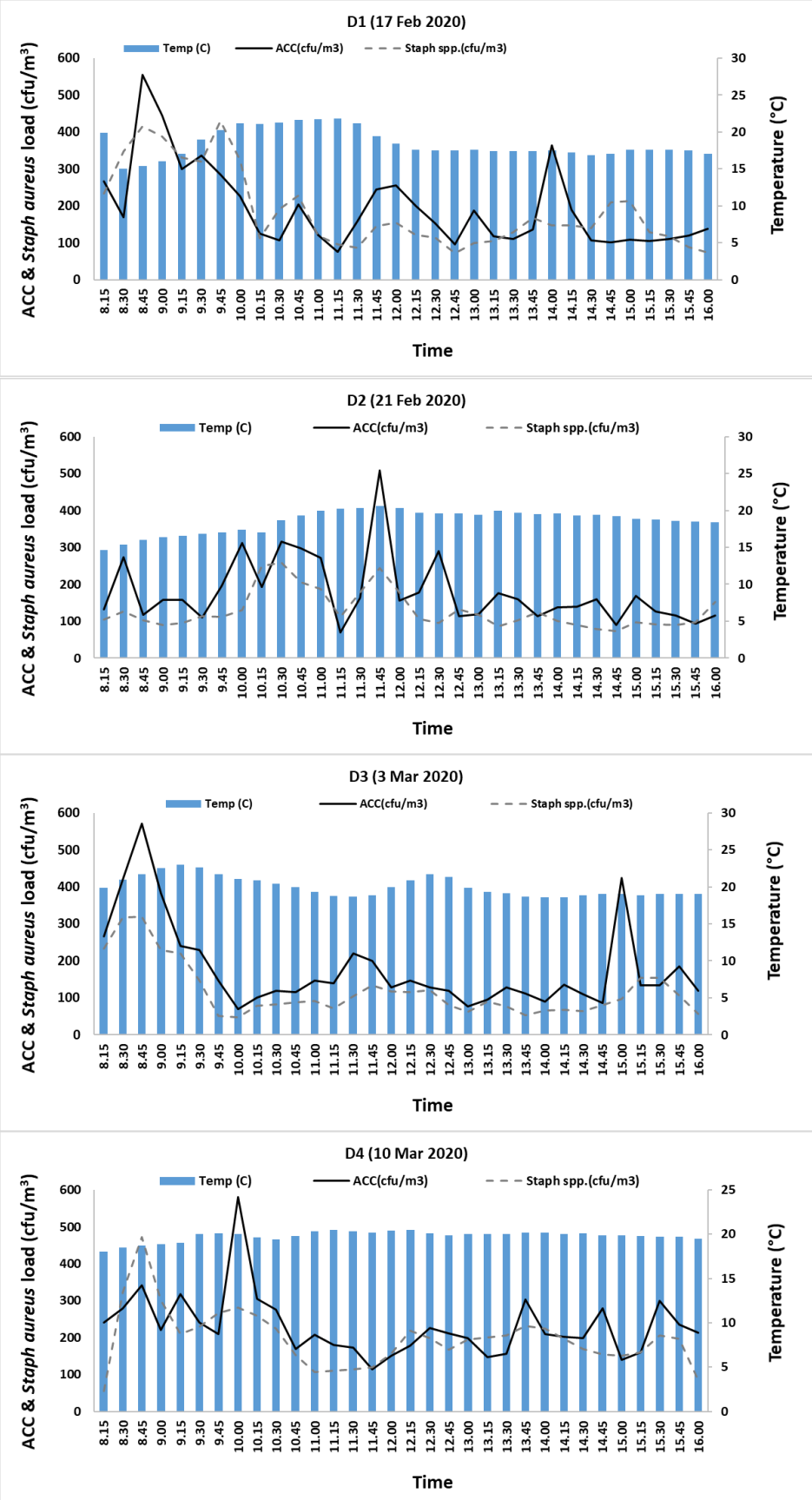

Figure 5:6 Fluctuation of the concentration of airborne microbial concentration (ACC) and the concentration of *Staphylococcus spp*. in the air (Staph spp) shown alongside the temperature (°C).

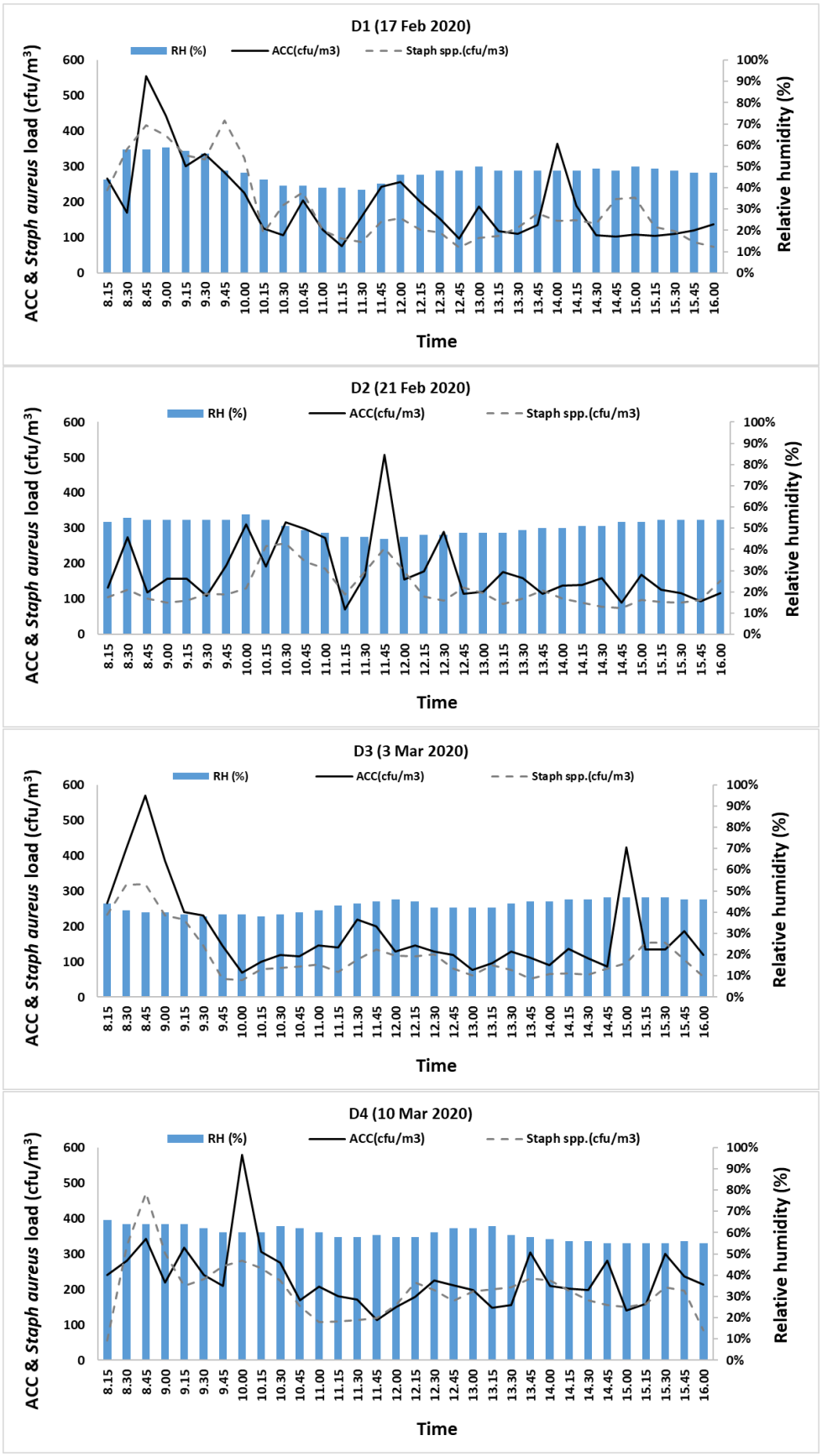

Figure 5:7: Fluctuation of the airborne microbial concentration (ACC) and the concentration of *Staphylococcus spp*. in the air (*Staph spp*) shown alongside the relative humidity (%).

The carbon dioxide level recorded within the four days was 644 ±115 ppm (433ppm -1168ppm) as shown in Table 5:2 and Figure 5:8. This result falls within the recommended level that is between 600 and 1000 ppm (Department of Health, 2007). The concentrations of ACC and *Staphylococcus spp*. in the air were moderately significant correlation with the CO_2 concentration (rho=0.34 [95%CI=0.17-0.49, n=128), *P*<0.001), and (rho=0.48 [95%CI=0.33-0.61), *P*<0.001, n=128), respectively Figure 5:5. The meta-analysis in Chapter 3 shows a similar finding where a moderately significant correlation was found for ACC r=0.53 (95% CI=0.40-0.64), P<0.001.

Particle mass concentration for PM2.5 and PM10 recorded within the four days were 19 ± 32 µg/m^3 (1 µg/m^3-151 µg/m^3), and 26 ± 44 µg/m^3 (1 µg/m^3-213 µg/m^3), respectively (Table 5:2 and Figure 5:9). The current UK outdoor guidelines are annual means of 25 µg/m^3 for PM2.5 and 40 µg/m^3 for PM10, while the most recent WHO recommendations from 2021 are only 5 µg/m^3 for PM2/5 and 10 µg/m^3 for PM10. Our values are close to and at times exceed the current UK outdoor guidelines and are above those of WHO. This suggests that the air quality isn't great.

There was no significant correlation between the ACC load and the particle mass concentration (2.5 µg/m^3) (rho=0.17, *P*= 0.058, n=128), while there was a significant correlation with the particle mass concentration (10 µg/m^3), (rho=0.19 [95%CI=0.02-0.35], *P*= 0.03, n=128) Figure 5:5. The meta-analysis reported that there was a moderately significant correlation between ACC and particle mass concentration ≤5 µg/m3 r=0.40 (95% CI= [0.04; 0.66]), P= 0.03, while ACC was not significantly correlated with particle mass concentration >5 µg/m3 r=0.23 (95% CI= [-0.07; 0.49]), P= 0.13. Furthermore, there was a significant correlation between *Staphylococcus spp*. and particle mass concentration (2.5 µg/m^3), (rho=0.30 [95%CI=0.13-0.45], *P*= 0.049, n=128) and particle mass concentration (10 µg/m^3). (rho=0.30 [95%CI=0.13-0.54], *P*= 0.049, n=128). Several of these factors can affect each other, so they should not be considered on their own but should always be taken along with others to provide more reliable results.

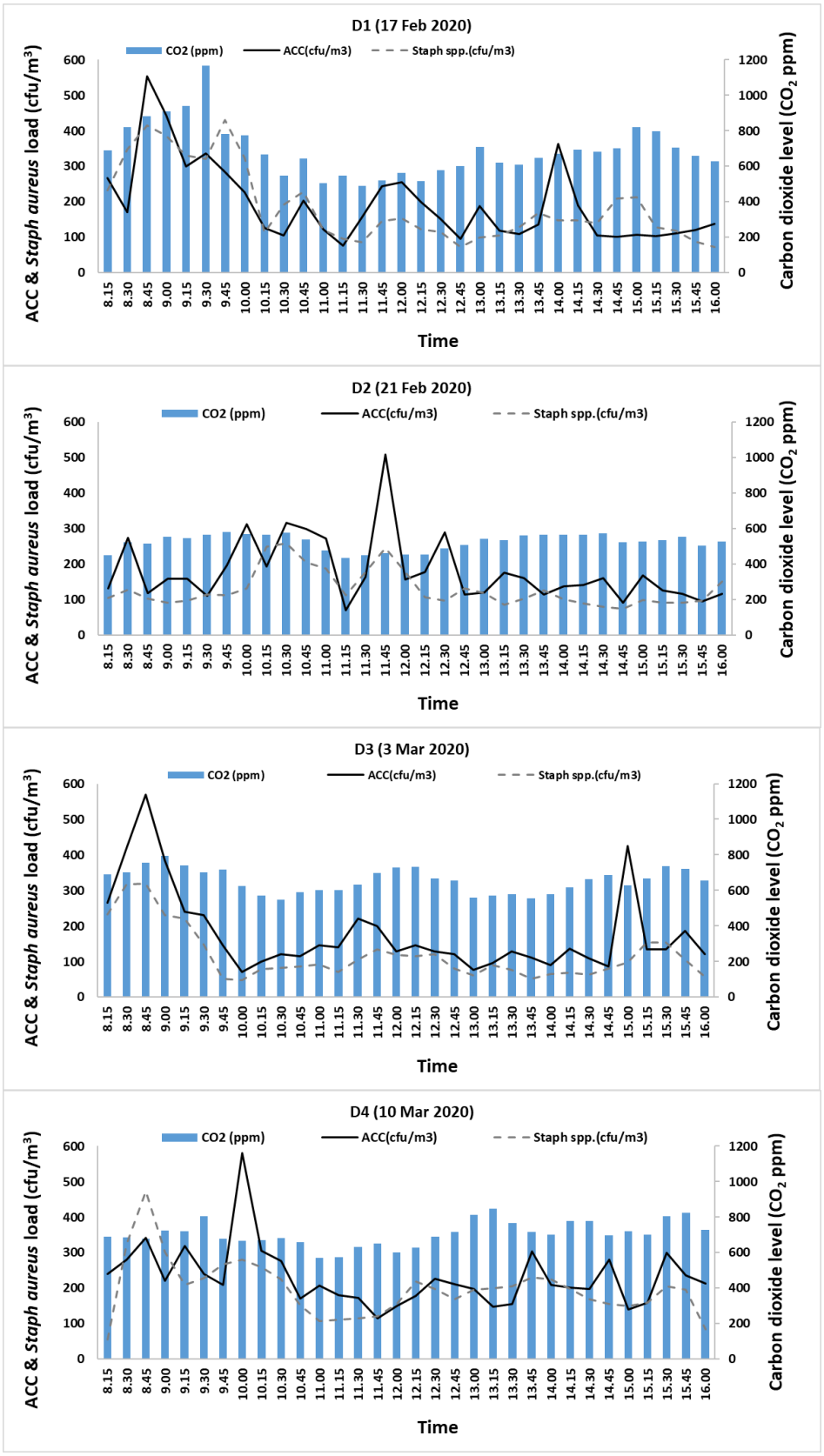

Figure 5:8: Fluctuation of the airborne microbial concentration (ACC) and the concentration of *Staphylococcus spp*. in the air (*Staph spp*) shown alongside the Carbon dioxide level (ppm).

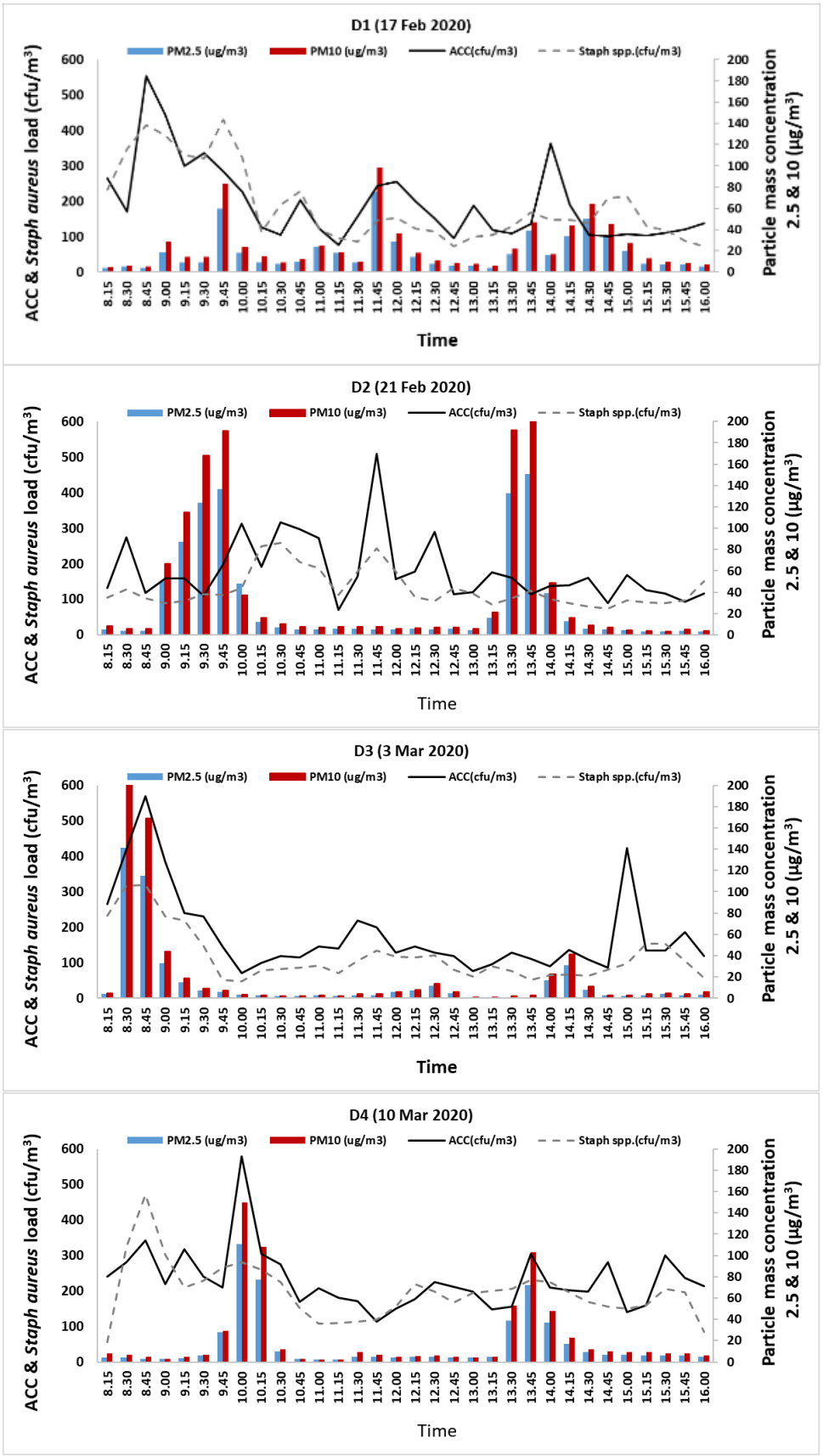

Figure 5:9 Fluctuation of the airborne microbial concentration (ACC) and the concentration of *Staphylococcus spp*. in the air (Staph spp) shown alongside the particle mass concentration (PM 2.5 µg/m^3) and (PM10 µg/m^3).

5.3.1.3 The relationship between airborne microbial or *Staphylococcus spp.* concentration and number of patients, visitors and HCWs over time

The average number of patients, visitors, and HCWs recorded per 15 minutes time-frame within the four-day study period were 51±7, 23 ± 21 and 10 ±12, respectively (Table 5:3). It is worth noting that due to room being prepared to admit COVID-19 patients, and fewer patients were being admitted to this room, there were a smaller number of patients and visitors on days 3-4 than on days 1-2.

Table 5:3 Descriptive statistics of presenting the level of patients, visitors and HCW.

	Day 1 (17 Feb 2020)	Day2 (21 Feb 2020)	Day3 (3 Mar 2020)	Day4 (10 Mar 2020)	Average
	Mean ± SD (Min-Max), Sample size				
Patient	54±7.93 (31-60), n=32	56±6.63 (45-60), n=32	49±6.55 (45-60), n=32	47±5.51 (60-45), n=32	51±7.37 (31-60), n=128
Visitor	31±29 (2-105), n=32	23±18 (2-82), n=32	21±16 (2-60), n=32	16±16 (2-45), n=32	23±21 (2-105), n=128
HCW	17±14.73 (1-60), n=22	17±12 (1-40), n=22	9±8 (1-35), n=22	15±13 (1-45), n=28	14±12 (1-60), n=94

Figure 5:10 shows the fluctuation in the number of patients, visitors and HCWs recorded per 15 minutes time-frame in eight hours' time series within the four days study.

The ACC load in the air was weakly negatively significantly correlated with the number of patients (rho= -0.18 [95% CI=0.01-0.35], *P*<0.037, n=128) while was not significantly correlated with the number of visitors and HCWs (Table 5:4 and Figure 5:11). The *Staphylococcus spp*. load in the air was not significantly correlated with neither the number of patients nor the number of visitors, while there was a significant correlation with HCWs (rho= 0.26 [95% CI=0.06-0.44], *P*<0.037, n=128). A previous study found no significant correlation between ACC and the number of HCWs (Hathway et al., 2013),

while another study found that there is a significant correlation between ACC and the number of HCWs (Fawcett, 2006).

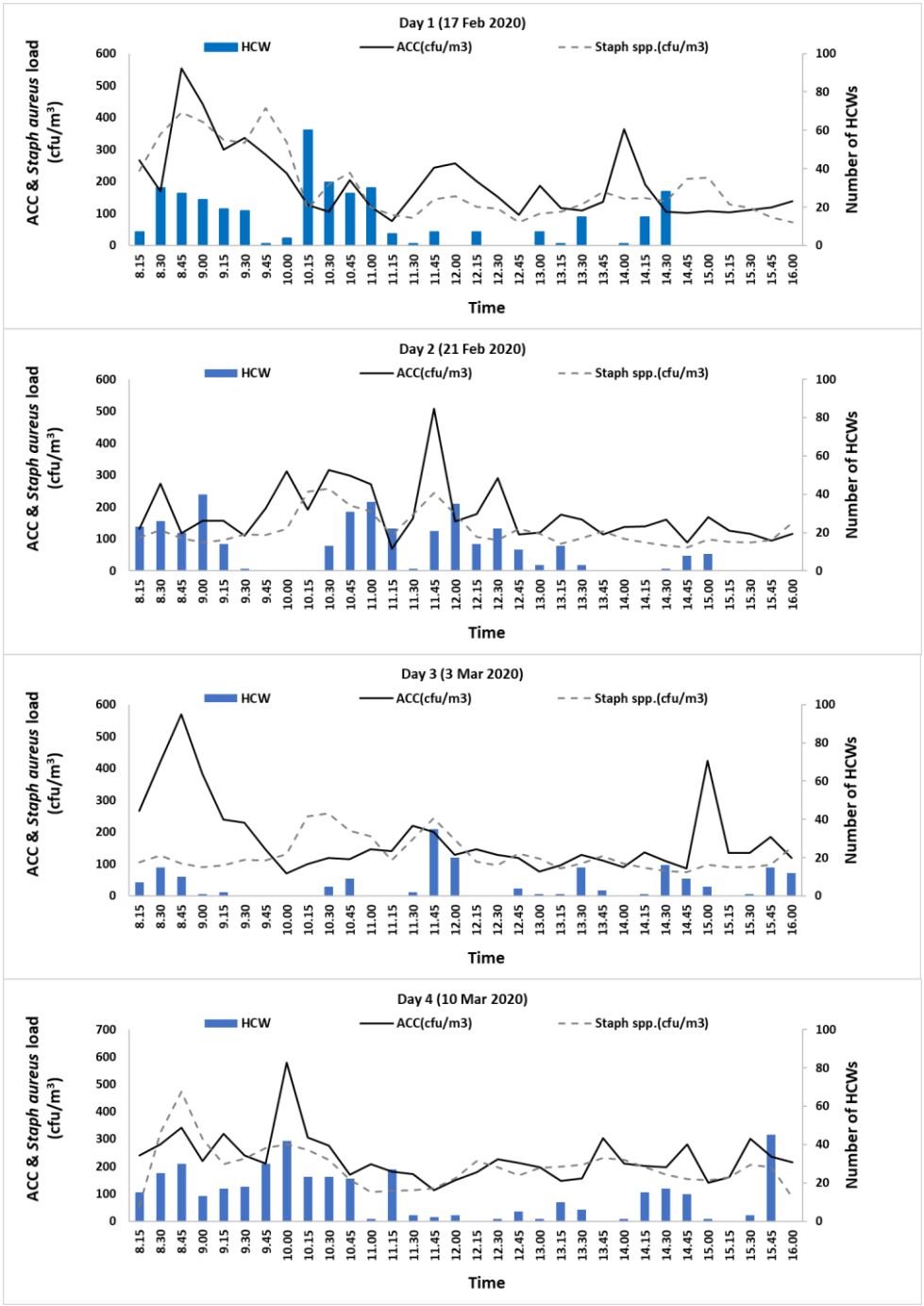

Figure 5:10 Fluctuation of the airborne microbial concentration (ACC) and the concentration of *Staphylococcus spp*. in the air (Staph spp) shown alongside the number of HCWs recorded per 15 minutes time-fram.

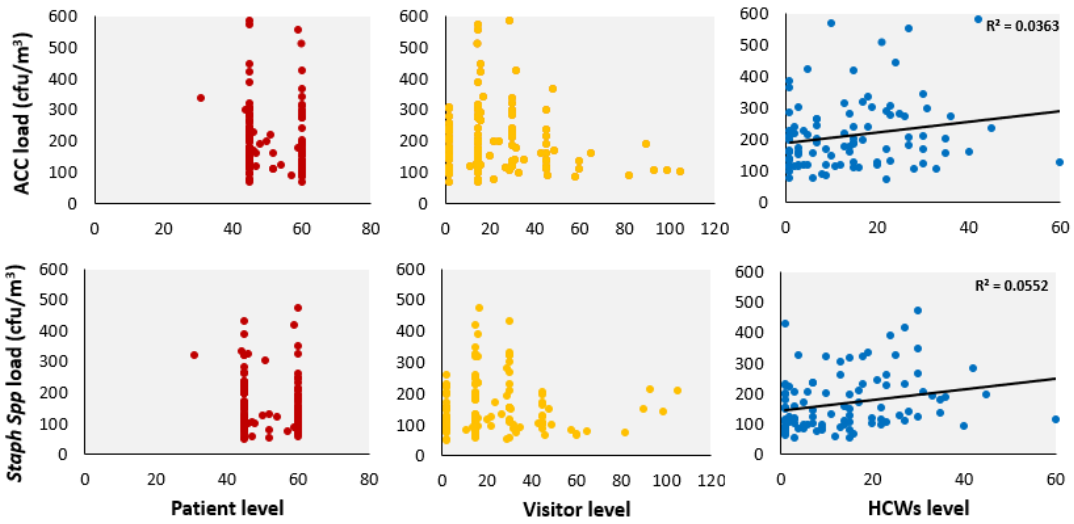

Figure 5:11 The correlation between airborne microbial concentration measured as ACC or *Staphylococcus spp*. (Staph spp) and the number of patients, visitors and healthcare workers (HCWs).

Table 5:4 Spearman's correlation coefficient between ACC load and *Staphylococcus spp*. load in the air and the number of patients, visitors and HCW over time.

	Spearman's rho	Patient	Visitor	HCW
ACC load in the air (cfu.m^{-3})	Correlation Coefficient (95% CI)	-0.18* (0.01-0.35)	-0.071	0.19
	Sig. (2-tailed)	0.04	0.43	0.06
	Sample size	128	128	94
Staph spp. load in the air (cfu.m^{-3})	Correlation Coefficient (95% CI)	-0.13	-0.05	0.26* (0.06-0.44)
	Sig. (2-tailed)	0.15	0.62	0.01
	Sample size	128	128	94

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

CI is the confidence interval of 95% of the data.

5.3.1.4 The relationship between airborne microbial or *Staphylococcus spp.* concentration and level of different healthcare activity over time

Table 5:5 shows the activity level (the number of HCWs multiplied by the duration time per 15 minutes frame of time). It presents the average time, the standard deviation and the minimum and maximum values for each activity. One easy example is the bed bathing activity; the first day shows a value of 15 ± 0 with n=2, meaning that the activity has occurred during two

time frames (episodes) with a level of 15 (the number of people present multiplied by the duration in minutes) for each episode.

Table 5:5: Descriptive statistics of the different activity levels of HCWs.

	Day 1 (17 Feb 2020)	Day2 (21 Feb 2020)	Day3 (3 Mar 2020)	Day4 (10 Mar 2020)	Average
	Mean ± SD (Min-Max), Sample size				
Talking	8±9.90 (0-15), n=2	10.67±8.33 (4-20), n=3	4.5±0.7 (4-5), n=2	9 n=1	8.25±6.36 (1-20), n=8
Meal	4.75±3.58 (1-11), n=8	3.15±3.39 (1-12), n=13	2.6±1.96 (1-6), n=10	2.3±1.25 (1-5), n=10	3.12±2.75 (1-12), n=41
Feeding	5 n=1	0 n=0	0 n=0	1.5±0.7 (1-2), n=2	2.67±2.08 (0-5), n=3
Medicine	10.33±6.62 (1-21), n=9	6.44±4.50 (1-13), n=9	8.75±4.65 (4-15), n=4	6.25±3.86 (2-10), n=4	8.12±5.28 (1-21), n=32
Nurse' observe	9.86±6.72 (1-15), n=7	6.22±3.03 (3-11), n=9	3.67±2.31 (1-5), n=3	5.4±2.88 (2-9), n=5	6.79±4.64 (1-15), n=24
Doctor' observe	18±15.47 (3-45), n=6	15.83±6.24 (11-26), n=6	10.67±7.57 (2-16), n=3	11.67±13.43 (2-27), n=3	15±10.88 (2-45), n=18
Bedmaking	4.5±4.95 (1-8), n=2	4.33±2.31 (3-7), n=3	8±9.90 (1-15), n=2	9±5.88 (1-15), n=8	7.33±5.62 (1-15), n=15
Bed bathing	15±0 n=2	6.6±6.5 (1-15), n=5	4.5±0.71 (4-5), n=2	12.7±10.68 (1-30), n=10	10.47±8.92 (1-30), n=19
Physiotherapy	0 n=0	5.33±5.13 (1-11), n=3	6.67±7.37 (1-15), n=3	0 n=0	6±5.73 (1-15), n=6
Cleaning	0 n=0	6±2.83 (4-8), n=2	6±2.12 (3-9), n=5	15.33±15.77 (1-45), n=6	10.31±11.37 (1-45), n=13

The airborne microbial concentrations measured for ACC and *Staphylococcus spp*. were not significantly correlated with any kind of activity except in the case of the *Staphylococcus spp*. load being significantly correlated with bed bathing (rho= 0.47 [95% CI=-0.01-0.77], *P*<0.04, n=19)

(Table 5:6 and Figure 5:12). These results agree with previous work, which found an increase in the airborne microbial load while bed-patient washing occurs (Hathway et al., 2013). Humans are considered to be the predominant source of bacteria in hospitals (Hiwar et al., 2021).

Table 5:6: Spearman's correlation coefficient between ACC load or *Staphylococcus spp*. load in the air and a different activity level of HCWs.

Activity level	Spearman's rho	Microorganisms load in the air (cfu.m^{-3})	
		ACC	*Staphylococcus spp*
Talking	Correlation Coefficient	-0.32	0.11
	Sig. (2-tailed)	0.44	0.80
	N	8	8
Meal	Correlation Coefficient	0.14	0.13
	Sig. (2-tailed)	0.37	0.43
	N	41	41
Medicine	Correlation Coefficient	0.23	0.19
	Sig. (2-tailed)	0.27	0.36
	N	26	26
Nurse's observe	Correlation Coefficient	-0.17	0.22
	Sig. (2-tailed)	0.44	0.30
	N	24	24
Doctor's observe	Correlation Coefficient	-0.20	-0.18
	Sig. (2-tailed)	0.42	0.49
	N	18	18
Bedmaking	Correlation Coefficient	0.30	-0.11
	Sig. (2-tailed)	0.28	0.70
	N	15	15
Bed bathing	Correlation Coefficient (95% CI)	0.29	.472* (-0.1-0.77)
	Sig. (2-tailed)	0.23	0.04
	N	19	19
Physiotherapy	Correlation Coefficient	-0.24	-0.32
	Sig. (2-tailed)	0.65	0.53
	N	6	6
Cleaning	Correlation Coefficient	0.25	0.29
	Sig. (2-tailed)	0.42	0.34
	N	13	13

* Correlation is significant at the 0.05 level (2-tailed).

CI is the confidence interval of 95% of the data.

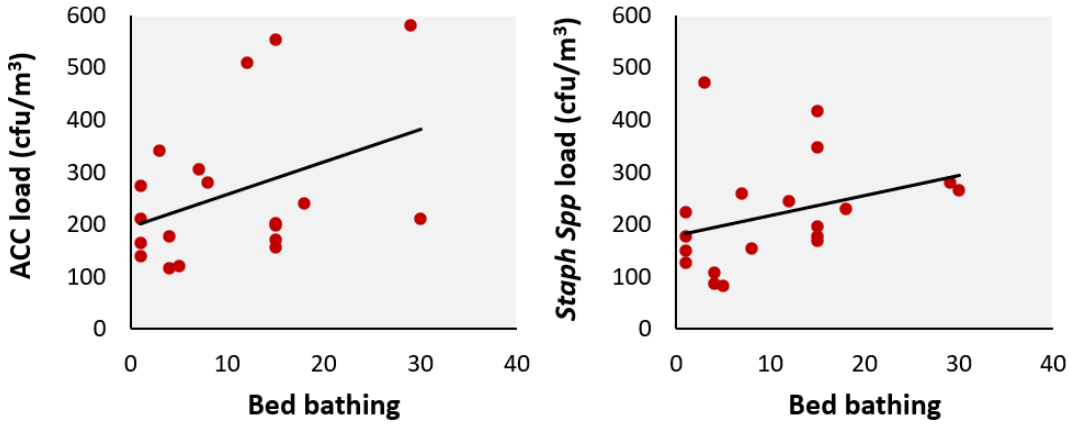

Figure 5:12 The correlation between airborne microbial concentration measured as ACC or *Staphylococcus spp.* (Staph) and Bedmaking, bed-patient washing.

5.3.1.5 The relationship between airborne microbial or *Staphylococcus spp.* concentration and natural ventilation

On the second day of sampling, the ward window was opened by 7cm in the morning, and then the gap was reduced to 2-3cm over the middle of the day, then it was closed mid-afternoon. During this day, the airborne concentration of both ACC and S. aureus was fluctuating, and after closing the window, they started to fall and were less variable. This can be due to a number of factors that influence the airborne concentration. The number of patients and HCWs is more likely to be the reason why this has occurred. However, there is not enough data to draw conclusions because there are not enough data to perform multi-variant analyses. Since opening (or closing) windows influence the ventilation of the room and may impact the results, more investigations regarding the ventilation rate in the ward setting and in controlled environments are still needed to obtain more reliable results and to better understand the impact of ventilation on the airborne microorganism's concentration (Figure 5:13).

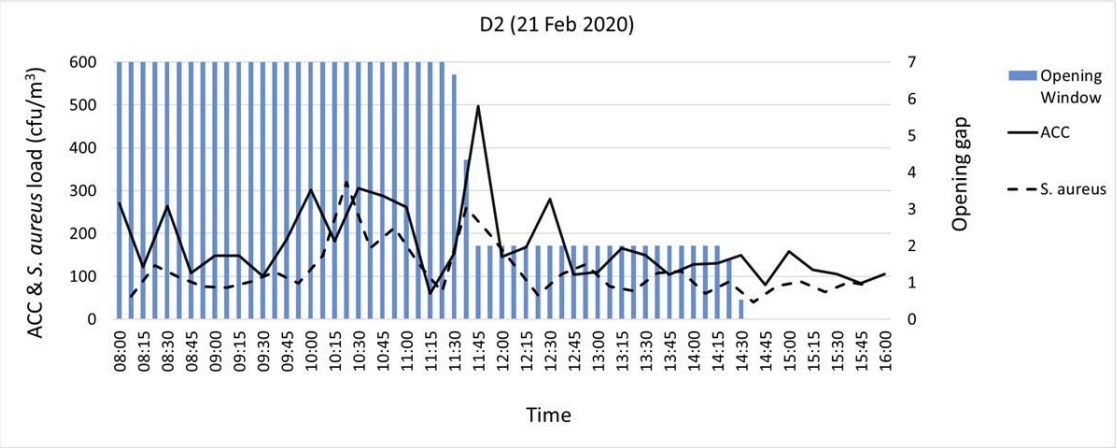

Figure 5:13 The effect of ventilation on the airborne concentration of ACC and *S. aureus*

5.3.2 Correlation between air and surface samples

5.3.2.1 Microorganisms load on surfaces in three-room wards

The descriptive statistics of microorganism concentrations (ACC and *Staphylococcus spp)* on five high touch surfaces in single-bed, four-beds and ten-beds in the respiratory ward are shown in Table 5:7. The average concentration of ACC on surfaces in a single bed was 0.92±0.58 cfu.cm^{-2} in the morning (9 am) and was 0.75±0.81 cfu.cm^{-2} in the evening (4 pm). The average concentration of *Staphylococcus spp* in the morning and in the evening were 0.39±0.37 cfu.cm^{-2} (42% of ACC) and 0.48±0.52 cfu.cm^{-2} (64% of ACC) respectively. In the four-beds bay, the ACC load on surfaces in the morning and the evening was 1.97±1.58 cfu.cm^{-2} and 1.24±1cfu.cm^{-2}, respectively, while the *Staphylococcus spp* load on surfaces in the morning and in the evening was 1.04±0.93 cfu.cm^{-2} (53% of ACC) and 0.78±0.64 cfu.cm^{-2} (63% of ACC), respectively. In the ten-beds bay, the ACC load on surfaces in the morning and the evening was 1.78±1.66 cfu.cm^{-2} and 1.90±2.36 cfu.cm^{-2}, respectively, while the *Staphylococcus spp* load on surfaces in the morning and the evening was 1.52±1.94 cfu.cm^{-2} (85% of ACC) and 1.31±1.43cfu.cm^{-2} (69% of ACC), respectively.

Table 5:7 The descriptive statistics of microbial contamination load on surfaces in three different ward rooms measured in the morning and evening.

		Day 1 (17 Feb 2020)	Day2 (21 Feb 2020)	Day3 (3 Mar 2020)	Day4 (10 Mar 2020)	Average
		Mean ± SD (Min-Max), Sample size				
Surface bioburden in a single bed room at 8am (cfu.cm^{-2})	ACC	0.92±0.58 (0.08-1.89), n=8	N/A	N/A	N/A	0.92±0.58 (0.08-1.89), n=8
	Staph spp.	0.39±0.37 (0.04-1.09), n=8	N/A	N/A	N/A	0.39±0.37 (0.04-1.09), n=8
Surface bioburden in a single bed room at 4pm (cfu.cm^{-2})	ACC	0.75±0.81 (0.04-2.3), n=7	N/A	N/A	N/A	0.75±0.81 (0.04-2.3), n=7
	Staph spp.	0.48±0.52 (0.04-1.47), n=20	N/A	N/A	N/A	0.48±0.52 (0.04-1.47), n=20
Surface bioburden in four-bed ward at 8am (cfu.cm^{-2})	ACC	1.57±1.13 (0.17-3.16), n=20	1.36±1.01 (0.04-3.62), n=20	2.85±2.12 (0.25-6.74), n=15	1.8±1.72 (0.08-5.85), n=20	1.97±1.58 (0.04-6.74), n=75
	Staph spp.	1.07±0.67 (0.13-2.74), n=20	1.03±0.73 (0.08-2.74), n=20	1.28±1.41 (0.08-5.43), n=15	0.78±0.88 (0.04-2.91), n=20	1.04±0.93 (0.04-5.43), n=75
Surface bioburden in four-bed ward at 4pm (cfu.cm^{-2})	ACC	1.49±1.04 (0.25-2.61), n=20	1.13±0.91 (0.04-3.16), n=20	1.58±1.19 (0.25-4.46), n=20	1.19±0.94 (0.25-3.28), n=15	1.24±1 (0.04-4.46), n=75
	Staph spp.	0.99±0.56 (0.04-2.23), n=20	0.62±0.45 (0.04-1.81), n=20	0.88±0.89 (0.04-2.95), n=20	0.58±0.50 (1-41), n=15	0.78±0.64 (0.04-2.95), n=75
Surface bioburden in ten-bed ward at 8am (cfu.cm^{-2})	ACC	1.55±1.19 (0.04-3.58), n=18	1.53±1.31 (0.04-5.05), n=17	2.74±2.18 (0.88-7.79), n=19	1.30±0.95 (0.38-3.75), n=19	1.78±1.66 (0.04-7.79), n=73
	Staph spp.	1.28±1.38 (0.21-5.01), n=18	1.24±1.02 (0.04-3.54), n=17	1.56±2.80 (0.13-8.21), n=19	1.11±1.08 (0.04-4.29), n=19	1.52±1.94 (0.04-8.21), n=73
Surface bioburden in ten-bed ward at 4pm (cfu.cm^{-2})	ACC	2.01±2.10 (0.38-8.42), n=15	1.27±1.07 (0.25-3.87), n=17	2.86±2.08 (0.04-8.29), n=19	1.47±2.72 (0.04-8.29), n=19	1.90±2.36 (0.04-8.42), n=70
	Staph spp.	1.31±1.53 (0.13-4.63), n=15	0.87±0.49 (0.08-2.11), n=17	1.64±1.28 (0.08-4.42), n=19	1.42±1.6 (0.08-5.01), n=19	1.31±1.43 (0.08-5.01), n=70

The concentrations of ACC on different surfaces and in different wards were of a similar order of magnitude and slightly lower than those found in a previous study which reported 3.46 cfu.cm^{-2} in the evening (four hours after using detergent-based cleaning) and 4.89 cfu.cm^{-2} in the morning of the following day (Bogusz et al., 2013), It was performed on different surfaces (bedside locker, left bedrail, overbed table and right bedrail) in a 30-bed ward.

A value of 2.5 cfu.cm^{-2} has been suggested to be the benchmark level for cleanliness of surfaces in hospital wards (Smith et al., 2018a). This value is therefore taken as a “pass-fail” criteria for evaluating the surface samples in the current study. Table 5:8 shows that the single-bed room has no failed values (≥ 2.5 cfu.cm^{-2}), and all the samples were marked as a pass (< 2.5 cfu.cm^{-2}). The four-bed ward had a lower percentage of failed samples in the evening (16%) 4 hours after cleaning than in the morning (32%). In the ten-bed ward, the percentage of failed samples were found to be the same (27%) in the morning and in the evening. Although the sampling was for only one day in the single-bed room, this result of zero fails could be due to the fact that HCWs can follow the hygiene procedures more easily than in larger rooms since it offers fewer distractions. In the larger rooms, HCWs may follow the hygiene recommendations and sanitise their hands upon entering but may also move from patient to patient and touch the surfaces at each station without sanitising due to being distracted or forgetting to use the sanitiser, leading to higher contamination.

Table 5:8: The percentage of failed samples according to the cleaning benchmark of (2.5 cfu.cm^{-2}) in different ward sizes in the morning and the evening.

	Day 1	Day2	Day3	Day4	Average
	%, (Total number of fails / Total number of sampling)				
Single bed room at 8am	0% (0/8)	N/A	N/A	N/A	0% (0/8)
Single bed room at 4pm	0% (0/7)	N/A	N/A	N/A	0% (0/7)
Four-bed ward at 8am	30% (6/20)	10% (2/20)	53% (8/15)	30% (6/20)	32% (24/75)
Four-bed ward at 4pm	15% (3/20)	10% (2/20)	25% (5/20)	13% (2/15)	16% (12/75)
Ten-bed ward at 8am	28% (5/18)	15% (3/17)	47% (9/19)	16% (3/19)	27% (27/73)
Ten-bed ward at 4pm	27% (4/15)	10% (2/17)	53% (10/19)	16% (3/19)	27% (19/70)

There was a phenomenon that appeared in our study and was also seen in the previous study (Bogusz et al., 2013). The percentage of ACC load on different surfaces and in different wards four hours after cleaning was the same as the following day before cleaning (85% ± 97%) while in the previous study, it was 71% (Figure 5:14). Also, the percentage of *Staphylococcus spp.* load in different surfaces and in different wards after four hours of cleaning was 80% ± 67% of the *Staphylococcus spp*. load in

the following morning before cleaning. This phenomenon can be explained by the natural decay of microorganisms or the decay that happened because the residual of cleaning fluid.

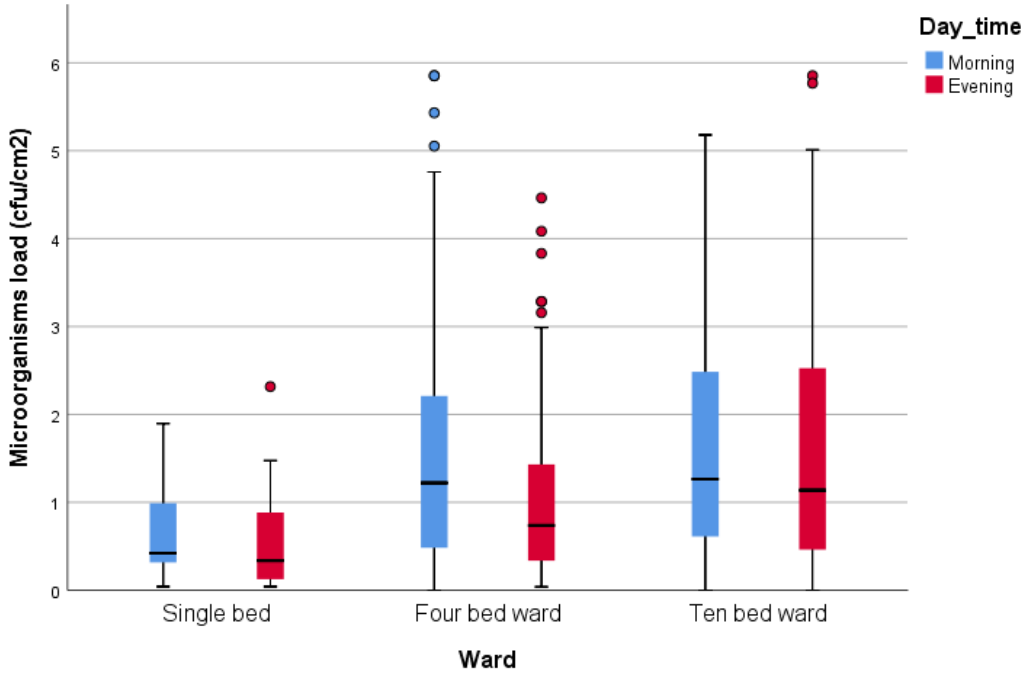

Figure 5:14 Microorganisms load measured through surface screening during the morning and evening for different size rooms

There was a significant difference between the microbial concentration measured as ACC on surfaces in the ten-bed ward and in the single-bed (*P*=0.016), while there was no significant difference between microbial concentration measured as ACC on surfaces in the ten-bed ward and in the four-bed ward (*P*=0.10). The concentration of *Staphylococcus spp.* on the surfaces in the ten-bed ward was significantly different from those in single-bed and four-bed wards *P*=0.011 and *P*=0.003, respectively (Table 5:9). The concentration of both ACC and *Staphylococcus spp.* in the ten-bed ward was significantly different from that in the single-bed, but the sample size of the single-bed room included only 15 samples taken on the same day (the first day). It is practically difficult to perform sampling several times for the same room since it affects the comfort and the convenience of the patient. There is still a need for further investigations to check whether this has a high confidence in the statistical difference.

Table 5:9: Correlations between surface microbial concentrations in three ward rooms in the morning and evening

	Microorganism's load (cfu.cm^{-2})			
	ACC		*Staphylococcus spp*	
(I) Ward	Ten-bed ward		Ten-bed ward	
(J) Ward	Single-bed room	Four-bed ward	Single bed	Four-bed ward
Mean Difference (I-J)	1.201*	0.398	1.062*	0.533*
Std. Error	0.433	0.196	0.364	0.159
Sig.	0.016	0.106	0.011	0.003
95% CI Lower Bound	0.181	-0.063	0.205	0.157
Upper Bound	2.220	0.859	1.920	0.908

* The mean difference is significant at the 0.05 level.

The high touch roof above the sink has the highest value of ACC load (3.23 ± 4.95, n=5) and of *Staphylococcus spp.* load (3.61± 4.66, n=3) compared with other types of surfaces (Table 5:10 and Figure 5:15). For the high hand touch surfaces, the concentration of ACC on the table (2.43 ± 1.52, n=50) was the highest level, and the left bed rail was the lowest where the bed table was the most frequently touched site.

The concentration of ACC and *Staphylococcus spp* on the right bed rail surfaces was found to be (1.05 ± 1.10, n=50) and (0.96 ± 1.84, n=50), respectively, which is slightly higher than that on the left bed rail (0.92 ± 0.83, n=50) and (0.53 ± 0.62, n=50), respectively. A previous study (Adams and Dancer, 2020), found that the left bed rail was slightly higher that the right bed rail one. This may be explained by difference in design and layout of the ward.

The *Staphylococcus spp* percentage of ACC load was found to be (62% ± 26%). Only one case (the high touch sink) shows that there is a higher concentration of *Staphylococcus spp* than that of ACC, which may occur by chance when sampling only a small part of the sink area.

Table 5:10 Microorganisms load on different surfaces in the hospital ward

		Microorganisms load (cfu.cm^{-2})					
	Surface	Mean	SD	Min	Max	Sample size	Percentage of (Staph spp./ACC)
ACC	Note	2.07	2.14	0.00	10.40	50	
	Tray	2.09	1.55	0.00	8.42	50	
	Table	2.43	1.52	0.00	5.85	50	
	Left bed rail	0.92	0.83	0.00	3.96	50	
	Right bed rail	1.05	1.10	0.00	5.18	50	
	IV	1.64	0.00	1.64	1.64	1	
	TS	0.74	0.98	0.04	1.43	2	
	Sink	1.58	1.50	0.13	5.85	16	
	High touch sink	3.23	4.95	0.08	11.79	5	
	Splash	2.34	3.37	0.00	10.32	8	
	Total	1.74	1.76	0.00	11.79	282	
Staph spp.	Note	1.36	1.27	0.08	5.01	48	66%
	Tray	1.16	0.89	0.00	4.63	47	55%
	Table	1.61	1.22	0.17	5.43	48	66%
	Left bed rail	0.53	0.62	0.00	2.95	47	57%
	Right bed rail	0.96	1.84	0.04	12.42	47	91%
	IV	0.46	.	0.46	0.46	1	28%
	TS	0.21	0.12	0.13	0.30	2	29%
	Sink	0.99	0.84	0.04	2.57	13	63%
	High touch sink	3.61	4.66	0.25	8.93	3	112%
	Splash	1.11	1.38	0.08	4.13	7	48%
	Total	1.14	1.34	0.00	12.42	263	62% ± 26%

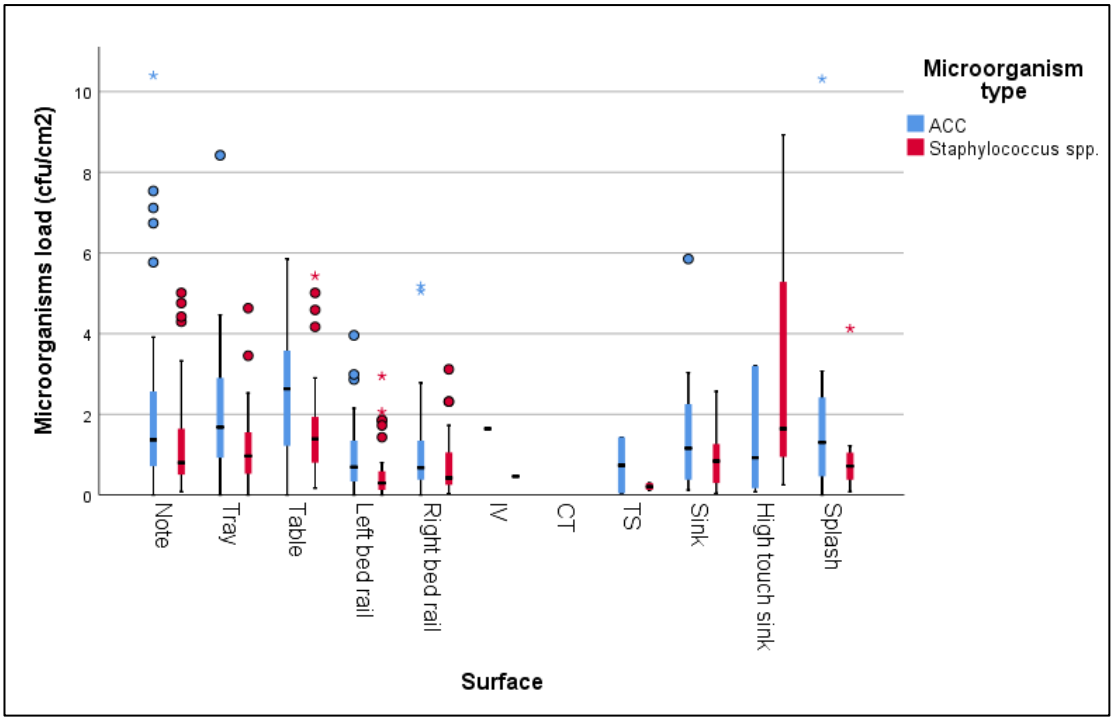

Figure 5:15 Microbial concentration measured as ACC and *Staphylococcus spp.* load on different surfaces across all three ward rooms.

Table 5:11 shows a comparison between the different surfaces, including the patient notes, tray, table and right and left bed rails. Other surfaces (IV, TS, sink, high touch sink, and the splash) were not considered due to the small sample size (number of samples) that is too low to be used for statistical comparison. It can be seen that the contamination at the notes, tray, and table is significantly higher than the contamination at the right bed rail (0.71 [CI 95% 0.03 – 1.39]), (0.63 [CI 95% -0.05 – 1.32]) and (1.02 [CI 95% 0.34– 1.71]), respectively. Also, the notes, tray, and table have a significantly higher contamination than the left bed rail (0.99 [CI 95% 0.31 – 1.67]), (0.91 [CI 95% 0.23 – 1.59]) and (1.30 [CI 95% 0.62– 1.98]), respectively.

Table 5:11 The mean difference of contamination between different types of surfaces in a hospital ward

(I) Surface	(J) Surface	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Note	Tray	0.08	0.21	1.00	-0.60	0.76
	Table	-0.31	0.21	0.91	-0.99	0.37
	Left bed rail	.99143*	0.21	0.00	0.31	1.67
	Right bed rail	.71318*	0.21	0.03	0.03	1.39
	IV	0.67	1.07	1.00	-2.73	4.07
	TS	1.25	0.76	0.83	-1.18	3.67

(I) Surface	(J) Surface	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	Sink	0.40	0.32	0.96	-0.60	1.41
	High touch sink	-1.65	0.55	0.08	-3.40	0.10
	Splash	-0.05	0.42	1.00	-1.37	1.27
Tray	Note	-0.08	0.21	1.00	-0.76	0.60
	Table	-0.39	0.21	0.72	-1.07	0.29
	Left bed rail	.91069*	0.22	0.00	0.23	1.59
	Right bed rail	0.63	0.22	0.10	-0.05	1.32
	IV	0.59	1.07	1.00	-2.81	3.99
	TS	1.17	0.76	0.88	-1.26	3.59
	Sink	0.32	0.32	0.99	-0.68	1.33
	High touch sink	-1.73	0.55	0.06	-3.49	0.02
	Splash	-0.13	0.42	1.00	-1.45	1.19
Table	Note	0.31	0.21	0.91	-0.37	0.99
	Tray	0.39	0.21	0.72	-0.29	1.07
	Left bed rail	1.30212*	0.21	0.00	0.62	1.98
	Right bed rail	1.02388*	0.21	0.00	0.34	1.71
	IV	0.98	1.07	1.00	-2.42	4.38
	TS	1.56	0.76	0.57	-0.87	3.99
	Sink	0.72	0.32	0.42	-0.29	1.72
	High touch sink	-1.34	0.55	0.31	-3.09	0.41
	Splash	0.26	0.42	1.00	-1.05	1.58
Left bed rail	Note	-.99143*	0.21	0.00	-1.67	-0.31
	Tray	-.91069*	0.22	0.00	-1.59	-0.23
	Table	-1.30212*	0.21	0.00	-1.98	-0.62
	Right bed rail	-0.28	0.22	0.96	-0.96	0.41
	IV	-0.32	1.07	1.00	-3.72	3.08
	TS	0.25	0.76	1.00	-2.17	2.68
	Sink	-0.59	0.32	0.70	-1.59	0.42
	High touch sink	-2.64513*	0.55	0.00	-4.40	-0.89
	Splash	-1.04	0.42	0.27	-2.36	0.28
Right bed rail	Note	-.71318*	0.21	0.03	-1.39	-0.03
	Tray	-0.63	0.22	0.10	-1.32	0.05
	Table	-1.02388*	0.21	0.00	-1.71	-0.34
	Left bed rail	0.28	0.22	0.96	-0.41	0.96
	IV	-0.05	1.07	1.00	-3.45	3.35
	TS	0.53	0.76	1.00	-1.90	2.96
	Sink	-0.31	0.32	0.99	-1.32	0.70
	High touch sink	-2.36688*	0.55	0.00	-4.12	-0.62
	Splash	-0.76	0.42	0.72	-2.08	0.56

* The mean difference is significant at the 0.05 level.

5.3.2.2 The relationship between the microbial deposition loss-rate coefficient air and hospital environments

The descriptive statistics of airborne deposited microorganisms (ACC and *Staphylococcus spp)* on open Petri dishes in the four-bed ward are shown in Table 5:12 and Figure 5:16. The average concentration of airborne deposited microbial concentration measured as ACC was 18±10 (cfu.plate.h^{-1}). This level of deposited ACC on open Petri dishes is higher than the previous study, which was conducted at the intensive care unit in a ten-bed bay in a hospital in Scotland (Smith et al., 2018a). Their results presented ACC using semi-quantitative sampling were 45% of sampling was found to be scanty growth (0-2 cfu. plate.h^{-1}), 48% was light growth (3-10 cfu.plate.h^{-1}) and only 2% was moderate growth (11-40 cfu.plate.h^{-1}). The *Staphylococcus spp.* was 8±6 (cfu.plate.h^{-1}), while the previous study did not measure its concentration.

Table 5:12 Descriptive statistics of passive air sampling.

	Microorganisms load in open petri dishes (cfu.plate^{-1}.h^{-1}) Mean ± SD (Min-Max), Sample size		Normalised microorganisms load (cfu.m^{-2}.h^{-1}) Mean ± SD (Min-Max), Sample size	
	ACC	*Staphylococcus spp.*	ACC	*Staphylococcus spp.*
Day 1 (17 Feb 2020)	18 ± 10 (4-44), n=16	14.5±7.5 (4-32), n=8	2662±1617 (786-6916) n=16	2281±1205 (1257-4951) n=8
Day2 (21 Feb 2020)	18±8 (4-32), n=16	8±5.5 (4-26), n=16	2668±889 (1500-4284) n=16	1280±866 (540-4020) n=16
Day3 (3 Mar 2020)	16±12 (4-48), n=16	6±5.5 (2-24), n=16	2368±1832 (629-7702) n=16	938±927 (314-3851) n=16
Day4 (10 Mar 2020)	18±8 (8-32), n=16	7±3.5 (2-14), n=16	2798±1200 (1415-5030) n=16	1140±574 (236-2044) n=16
Average	18±10 (4-48), n=64	8±6 (2-32), n=56	2624±1385 (629-7702) n=64	1410±893 (236-4951) n=56

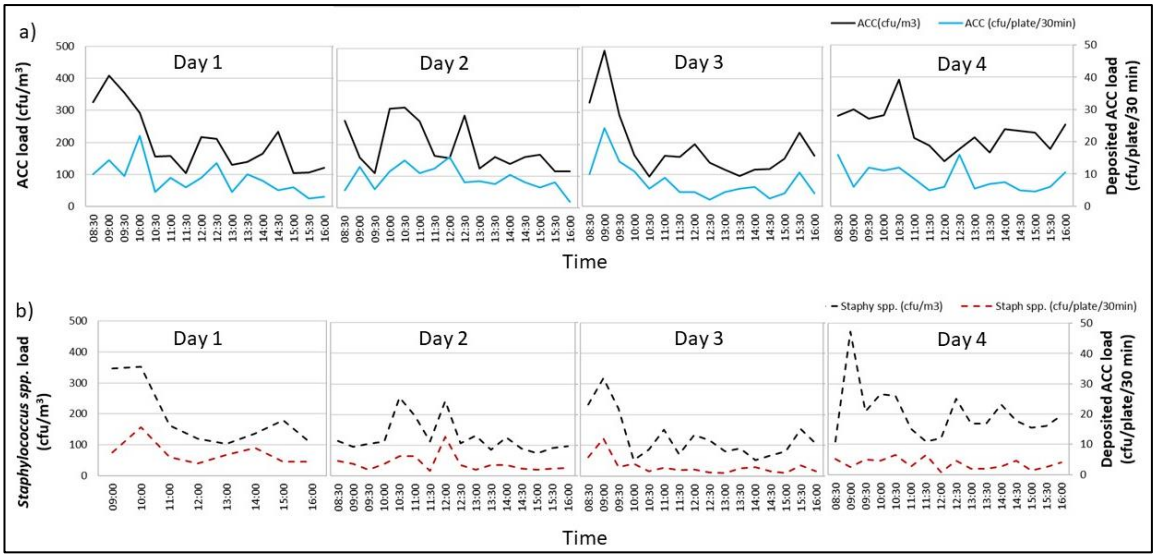

Figure 5:16: Active and passive air sampling results for four days on the time series a) microbial concentration measured as ACC and b) *Staphylococcus spp.* load.

Assuming a well-mixed environment, the relationship between quantitative microorganisms in the air measured by active sampling and on surfaces measured by passive sampling can be described using equation (4.7) which is discussed in chapter 4. Since the room is shaped as a cuboid, the volume of the room divided by the area of the floor (assuming that all particles deposit on the floor) equals the height of the room (H).

$$\lambda_{d.f} = \frac{C_{s,f}}{C\ H} \qquad (5.2)$$

Where, H is the height of the room in the ward (3m). Figure 17 presents $\lambda_{d.f}$ as the slope of the line fit for the scatter plot of $C_{s,f}/H$ vs C.

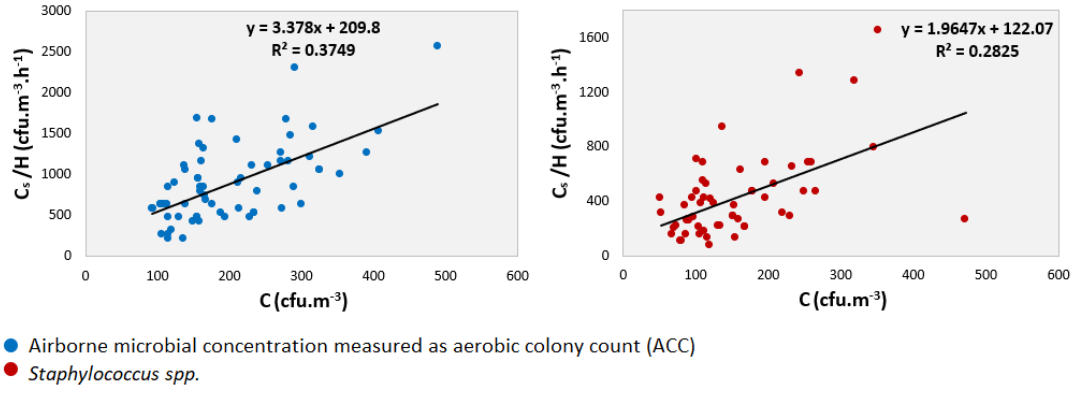

Figure 5:17: The scatter plot of the concentration of microorganisms on Petri dishes divided by the room height ($C_{s,f}/H$ cfu.m^{-3}.h^{-1}) against the mean indoor airborne microorganisms concentration (C cfu.m^{-3}).

The results show a high deposition rate coefficient in the hospital for both ACC and Staphylococcus spp. 3.38 (h^{-1}) and 1.96 (h^{-1}) respectively. The deposition rate found here is higher than suggested in previous works 0.17 ± 0.06 (h^{-1}) for the particle size 0.65 µm in a controlled environment, 0.88 (h^{-1}) for particle size 1-2 µm, 1.61 (h^{-1}) for particle size 2-5 µm, while it was lower than the 6.12 (h^{-1}) value reported for particle size 9-10 µm (Thatcher, et al., 2002; Lai, 2002; Howard-Reed et al., 2003; Tran et al., 2017). This may be due to the presence of larger-sized particles that result in greater deposition, or it is also possible that these larger particles are picked up by the settle plates but not the air sampler, which would further confound the results.

A previous study found that the microbial carrying particle size is (12-15 µm), which is associated with skin squama (Pankhurst et al., 2011). The deposition rate coefficient can be calculated according to equation 5.2 for each time frame of passive sampling (30 minutes) to get the results for ACC 5±2 (2-11) and for *Staphylococcus spp*. 3±2 (1-8). The surface air ratio for the ACC was 14 ± 6 (5-32) and for *Staphylococcus spp*. was 9 ± 5 (2-24). It is worth mentioning that equation 5.2 does not consider the effect of ventilation and airflow or the particle size. Also, it assumes that the deposition is only vertical and that there are no particles depositing horizontally on the walls or upwards onto the ceiling.

The loss rate due to deposition is not constant at each time frame as it can be affected by several factors. There is a significant negative correlation for $\lambda_{d,f}$ of ACC and $\lambda_{d,f}$ of *Staphylococcus spp* with CO_2 rho= -0.32, P= 0.009, n=64, rho= -0.38, P= 0.003, n=56, respectively. (Table 5:13) The value of $\lambda_{d,f}$ of *Staphylococcus spp* has a negative significant correlation with the day of sampling (rho= -0.53, P= 0.0, n=56) and a positive significant correlation with the number of patients (rho= 0.344, P= 0.009, n=56). For statistical reasons, any episode (activity) that has occurred less than ten times was not included. As it is challenging to separate the different factors that affect deposition, further investigations should be done in a controlled environment

to better quantify the effect of different particle sizes and airflow parameters on the relationship between microorganisms in the air and on surfaces.

Table 5:13 Spearman rho correlation coefficients of environmental parameters with ACC and *Staphylococcus spp.*

		loss-rate coefficient $\lambda_{d,f}$	
	Spearman's rho	ACC	*Staphylococcus spp*
Time	Correlation Coefficient	-0.164	-0.137
	Sig. (2-tailed)	0.194	0.313
	N	64	56
Day	Correlation Coefficient	-0.175	-.530**
	Sig. (2-tailed)	0.166	0
	N	64	56
Patient	Correlation Coefficient	0.092	.344**
	Sig. (2-tailed)	0.471	0.009
	N	64	56
Visitor	Correlation Coefficient	0	0.12
	Sig. (2-tailed)	0.997	0.379
	N	64	56
HCW	Correlation Coefficient	-0.254	-0.02
	Sig. (2-tailed)	0.056	0.89
	N	57	50
Meal	Correlation Coefficient	-0.009	0.038
	Sig. (2-tailed)	0.957	0.832
	N	36	33
Medicine	Correlation Coefficient	0.002	0.314
	Sig. (2-tailed)	0.995	0.236
	N	20	16
Nurse's observe.	Correlation Coefficient	-0.43	-0.345
	Sig. (2-tailed)	0.066	0.191
	N	19	16
Doctor's observe.	Correlation Coefficient	-0.203	-0.028
	Sig. (2-tailed)	0.487	0.931
	N	14	12
Bedmaking	Correlation Coefficient	-0.455	0.183
	Sig. (2-tailed)	0.187	0.637
	N	10	9
Bed bathing	Correlation Coefficient	-0.069	-0.055
	Sig. (2-tailed)	0.823	0.858
	N	13	13
Cleaning	Correlation Coefficient	-0.333	-0.144
	Sig. (2-tailed)	0.318	0.673
	N	11	11
PM2.5	Correlation Coefficient	0.093	0.126
	Sig. (2-tailed)	0.465	0.355
	N	64	56

		loss-rate coefficient $\lambda_{d,f}$	
	Spearman's rho	ACC	*Staphylococcus spp*
PM10	Correlation Coefficient	0.076	0.139
	Sig. (2-tailed)	0.548	0.306
	N	64	56
Temperature	Correlation Coefficient	0.209	-0.253
	Sig. (2-tailed)	0.097	0.06
	N	64	56
Relative humidity	Correlation Coefficient	-0.17	-0.191
	Sig. (2-tailed)	0.178	0.158
	N	64	56
Carbon dioxide	Correlation Coefficient	-.324**	-.385**
	Sig. (2-tailed)	0.009	0.003
	N	64	56

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Passive sampling has been suggested as a cheap and more approachable method to measure the indoor air microbial load (Pasquarella et al., 2000). According to equation (5.3), we can provide a mathematical equation that relates airborne microorganism concentration and surface load, which could be used to calculate airborne microorganisms as cfu/m^3 when using a passive sampling method (open Petri dishes). The mean of $\lambda_{d,f}$ that was found in a hospital environment is equal to 3.38 (h^{-1}). cfu is the number of microorganisms on Petri dishes as colony form unit), a is surface area in cm^2 and t is time in h^{-1}.

$$C = \frac{cfu}{\frac{a}{10000}\ t\ H\ 3.38} \qquad (5.3),$$

Table 5:14 shows the formula in each most common standard petri dishes diameter. It presents the concentration of airborne microorganisms on petri dishes with different sizes during a sampling period of one hour.

Table 5:14 The formula in each common standard Petri dishes diameter of one-hour sampling.

	100mmØ (a= 78.53 m^2)	90mmØ (a= 63.62 cm^2)	60mmØ (a= 28.27 cm^2)	55mmØ (a= 23.76 cm^2)
C =	C/ 0.080	C/0.065	C/0.029	C/0.024

C is the colony form unit in the Petri dishes, a is the area of the Petri dishes and C_{ia} is the mean indoor airborne microorganisms concentration ($cfu.m^{-3}$).

5.3.2.3 Contribution of airborne microorganisms to the surface bioburden

To quantify the contribution of airborne microorganisms to the surface microbial load in the healthcare environment, we compared the accumulated bioburden load on surfaces measured directly via contact plates and via passive settle plates. This was determined for all four days of sampling in the four-bed ward over the period from 12 pm (cleaning time assuming that the load is zero at this point) to 4 pm when the evening surface sampling was performed (Table 5:15). The contribution of the airborne microorganism load to the total surface load was calculated to be 65% ± 18% (range 39% - 82%) for the first four hours after cleaning.

Table 5:15 The contribution of airborne ACC on surface contamination in a four-bed ward for 4 hours (12 pm - 4 pm).

	Day 1	Day 2	Day 3	Day 4	Average
	mean ± SD (Min-Max), sample size				
Surface (cfu/cm^2/4h)	1.49 ± 1.04 (0 - 4.08), n=20	0.83 ± 1.05 (0 - 3.62), n=20	1.58 ± 1.19 (0.25 - 4.46), n=20	0.99 ± 0.94 (0.25 -2.99), n=16	1.36 ± 1.07 (0 - 4.46), n=75
Petri dishes (cfu/cm^2/4h)	0.83	0.93	0.62	0.98	0.7
Contribution	56%	82%	39%	82%	65% ± 18% (39% - 82%)

5.4 Summary

This chapter investigates the correlation between airborne ACC and *Staphylococcus spp.* with the hospital environment parameters, including air

quality, human presence, and HCWs activities. The surface bioburden of different wards was studied, and several parameters were assessed in terms of their influence on the deposited microorganisms. The chapter also presents a mathematical equation to describe airborne microorganism concentration and surface contamination in terms of cfu/m^3 to enable the comparison in concentration using passive and active sampling methods. The major findings of this chapter can be summarised as follows:

- The average concentration of airborne microbial concentration measured in the four-bed ward over a four-day period for ACC was 196±103, and it was significantly correlated with *Staphylococcus spp,* which represented (86%±35%) of ACC. This indicates that the sampling results support the previous findings in the literature (in similar settings).
- IAQ parameters were studied and recorded where temperature (19±2°C), relative humidity (50% ± 7%), Carbon dioxide level (644 ±115 ppm), particle mass concentration 2.5 µg/m^3 (19 ± 32 µg/m^3), and particle mass concentration 10 µg/m^3 (26 ± 44 µg/m^3). These values for temperature, humidity and CO_2 suggest a good indoor environment however the particle mass slightly exceeds the current UK outdoor and WHO guidelines, which means that it needs to be improved.
- The airborne ACC load was significantly correlated with relative humidity, CO_2 and with particle mass concentration 10 µg/m^3 while not significantly correlated with temperature, particle mass concentration 2.5 µg/m^3. The airborne *Staphylococcus spp* load was significantly correlated with relative humidity, CO2, and with both particle mass concentrations 2.5 µg/m^3 and 10 µg/m^3, while not significantly correlated with temperature. This indicates that controlling IAQ parameters could lead to reducing airborne bioburden which can potentially reduce the airborne infection risk.
- The average number of patients, visitors and HCWs recorded per 15 minutes time frame within the four-day period of study were represented as the number of people present, multiplied by the duration in minutes and were found to be 51±7, 23 ± 21 and 10 ±12, respectively. Since people exhale CO_2, data on their presence and density can be potentially useful in determining appropriate protocols for visitors.

- The ACC load in the air had a weak negative significant correlation with the number of patients, while it was not significantly correlated with the number of visitors and HCWs. On the other hand, the *Staphylococcus spp*. load in the air was not significantly correlated with neither the number of patients nor the number of visitors, while it was significantly correlated with HCWs. This may be due to the skin shedding of HCWs resulting in more *Staphylococcus spp*.
- The ACC in air was not significantly correlated with any of the activities while the *Staphylococcus spp.* load was only significantly correlated with bed bathing.
- The value of 2.5 cfu.cm^{-2} was suggested to be the benchmark level for cleaning the surfaces. A sample that has a value (≥ 2.5 cfu.cm^{-2}) is assumed a "failed" value and a sample with a value (< 2.5 cfu.cm^{-2}) is considered as "pass". All the samples in the single-bed room were passed. The four-bed ward had 16% failed samples four hours after cleaning, and 32% failed samples in the morning of the next day. The ten-bed ward had 27% failed samples both in the evening and the morning of the next day. This supports previous findings in the literature and confirms that a single-bed room offers a lower surface infection risk.
- The deposition on surfaces was studied, and the contamination at the note, tray, and table was significantly higher than the contamination at the right bed rail and the left bed rail.
- The average concentration of deposited ACC on open Petri dishes was 18±10 (cfu.plate.h^{-1}).
- A mathematical equation that relates airborne microorganism concentration and the surface load was provided to be used to calculate airborne microorganisms as cfu/m^3 when using passive sampling. This will provide a cheap and accessible approach to measure the airborne concentration.
- The contribution of the airborne microorganism load to the total surface load was calculated to be 65%± 18 after four hours of cleaning. This indicates that controlling airborne transmission risk will reduce the surface risk as well.

- With the large number of factors and the fluctuation in some of the results, more data needs to be collected in a controlled environment to obtain more reliable results of deposition coefficient and to better understand the influence of these factors in a real-world setting.

6 A Multiplate passive air sampler to measure deposition rate of airborne microorganisms overtime

6.1 Introduction

Airborne microorganisms are an integral part of non-sterile environments, some of which may be pathogenic to humans (Morawska et al., 2020c). As demonstrated in chapter 5, the passive sampling technique is essential in research to measure the deposition rate of microbes with regards to different settings (geometry design, temperature, relative humidity, ventilation regime and rate etc.) of controlled environments. It is also affordable, easy to handle, and can be used to measure the concentration of airborne microorganisms. Previous work shows that passive air sampling results can show the relation between air contamination and infection risk and could possibly be used as a proxy for infection risk (Smith et al., 2018b).

Passive sampling provides an aggregate sample over a period of time. In many real-world environments, discrete time periods can be measured by manually opening and closing Petri-dishes. However, sampling for long periods of time (typically at least 30 min) is needed to get a reliable count on an agar plate so collecting multiple samples can be time consuming. On the other hand, in a controlled chamber setting where the airborne microbial concentration is deliberately introduced and can be higher, higher deposited counts for deposition are to be expected and sample times can be shorter. Previous studies measured the spatial variation in deposition under a few different room geometries in comparison with a CFD model using *Staphylococcus aureus* (King et al., 2013) and a salt tracer as a surrogate tracer capable of representing *Staphylococcus aureus* bioaerosol (King et al., 2017a); both experiments were done in a controlled environment and were an aggregate sample rather than measuring the change over time.

The variation of deposition with time in these previous chamber studies was not investigated due to the lack of a device to collect the microorganisms at intervals of time during the whole experiment. For this reason, the results could not show the full time-cycle curve leading to a certain concentration or to measure variability during steady conditions. To reliably obtain this curve, measurements should be taken at close time intervals without human

intervention. In a controlled chamber with an aerosolised microorganism that could be pathogenic, it is not possible for a person to enter the room. In a real-world environment it can also be challenging to physically access the room and change the plates manually in a timely manner without causing inconvenience to the patient or for safety reasons where a patient may have a contagious disease. This makes it difficult to capture the transient effects without employing an automated method. According to our knowledge, there is no commercial equipment that can expose the settle plates to the air for a defined period before being covered.

This chapter aims to develop and test a novel configurable device that can expose a plate to air for a pre-determined interval, cover it, and autonomously expose a different one. This enables passive sampling in a controlled environment with a clear and accurate time curve showing the deposition of airborne microorganisms.

6.2 Concept and design

The main idea of the Automated Multiplate Passive Air Sampling (AMPAS) device is to enable sampling for several discrete time intervals over a defined period of time. It has to be programmable so that it can be used flexibly and to be able to adapt to different requirements and different settings. It must be able to expose an agar plate to the microorganisms in air at a pre-determined time for a pre-determined period and then cover it to make sure it is no longer exposed to air. The device must also be able to perform sampling on multiple plates to enable the collection of data safely with minimal human intervention.

To achieve this, a configurable microcontroller needs to be used to enable the user to program the device and set the timers to the required settings. It also requires the use of motors to enable moving or rotating the plates. This means that a battery or a power source is also needed to power the electronic and electrical components. The schematic in Figure 6:1 shows the connections between the microcontroller, the H-bridge, the power source, and the stepper motor. It also indicates the pins used for each connection. Furthermore, the hardware used must be able to prevent air from reaching to

plates and to protect them from any further contamination once they are covered.

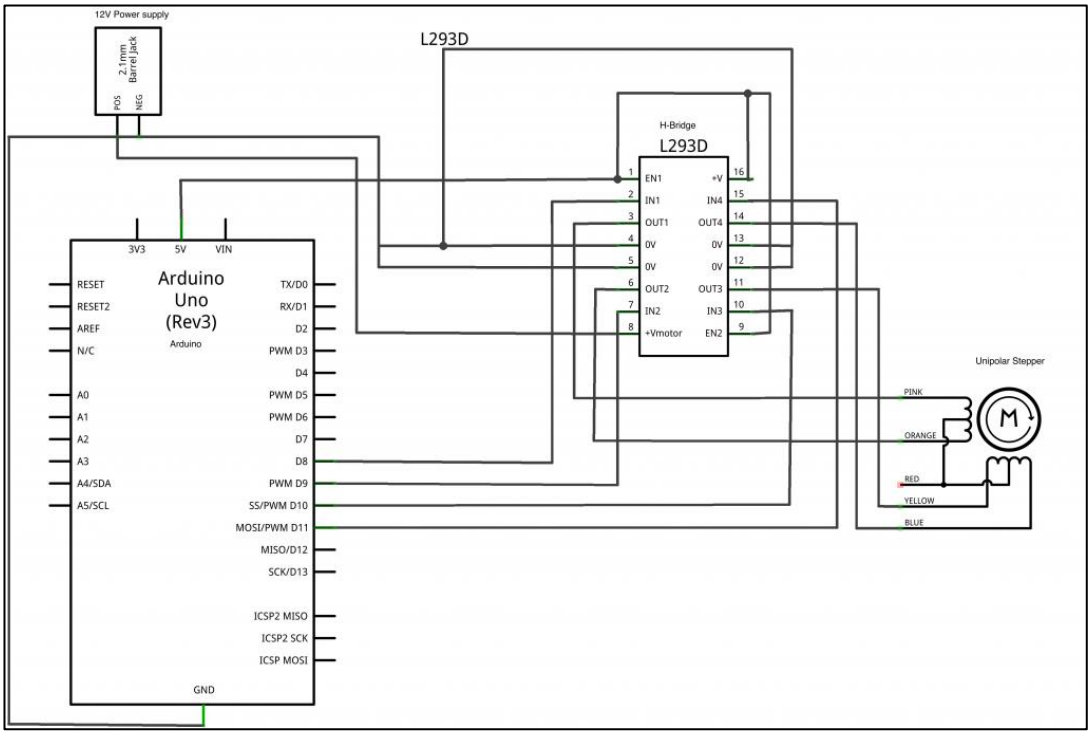

Figure 6:1 Schematic drawing of an h-bridge and stepper motor connected to an Arduino, with the control wires added (Computing, 2020).

6.3 Hardware materials and assembly

The AMPAS device designed and built during this study is shown in Figure 6:2. Assembly and operation are summarised in the following sections.

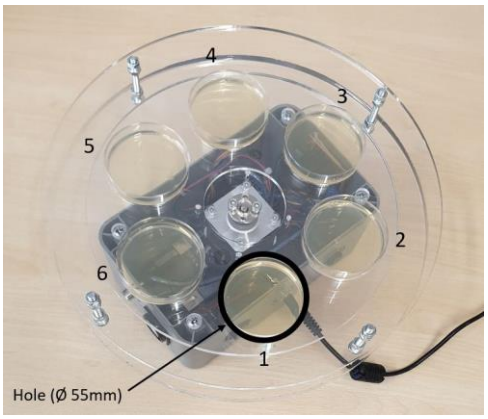

Figure 6:2 Automated multiplate passive air sampler (AMPAS).

6.3.1 Sampler base

An outdoor junction box (W180 × D110 × H100 mm) is used to store and protect the modules and cables from dust and water; it also works as a base to hold the trays and has a port (2.1 mm) for external DC power source. This box also contains an On/Off switch, an LED power indicator, safety fuse and a USB port for the programming and configuration of the microcontroller inside.

6.3.2 Microcontroller

A programmable microcontroller (Elegoo mega 2560) board to allow flexible and precise control over the stepper motor that rotates the middle trays to allow a specific plate to be exposed to air for a given period is used. A C-program reads the input ports and controls the output ports to set the rotation speed and direction of the stepper motor by utilising the H-Bridge circuit Figure 6:3.

6.3.3 Motor driver module

A Neuftech L298N Dual Channel H-Bridge is used which is an electronic circuit to alternate the polarity of the voltage and enable the stepper motor to rotate in each direction. The microcontroller sets the direction, the number of steps and the speed of the stepper motor Figure 6:3.

6.3.4 Stepper motor

The NEMA 17 bipolar stepper motor with a 0.9° step angle, holding torque (36Ncm), dimensions (W42 x D42 x H4mm), shaft diameter: 5mm, shaft length: 22mm, D-cut length: 15mm and weight (280 g), 0.9A rated current and 5.4V voltage. The stepper motor receives signals from the microcontroller through the H-Bridge and rotates accordingly. The motor is held by four screws through the base tray. The 5mm shaft of the stepper motor is attached to the middle tray using a coupler motor connector Figure 6:3.

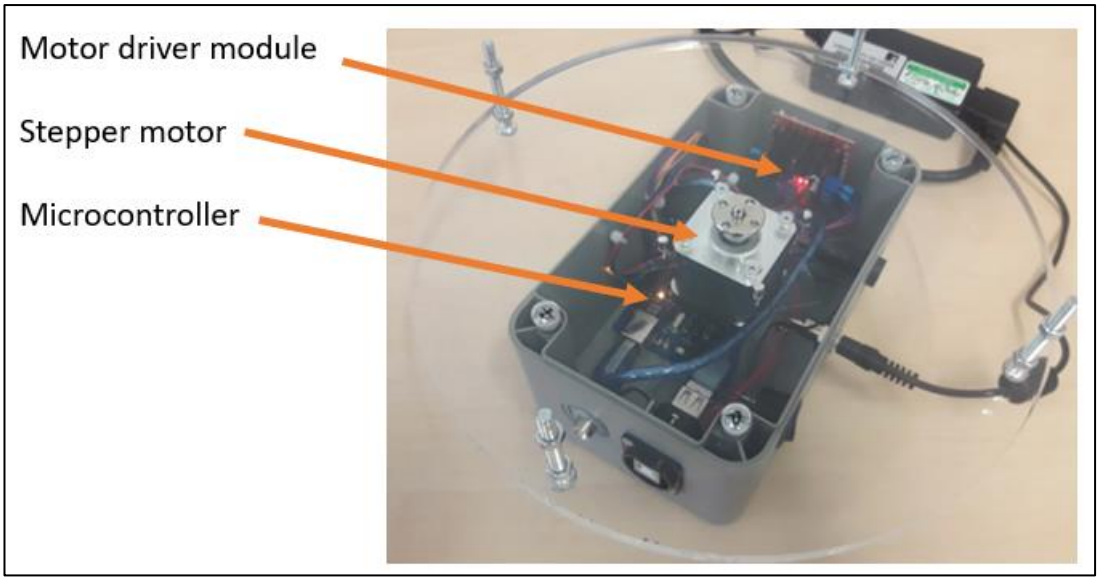

Figure 6:3: Assembly of Automated multiplate passive air sampler (AMPAS) component.

6.3.5 Trays

There are four transparent acrylic sheets used as trays, a first tray (base) is Ø 260mm circle sheet (10mm thick) with a small hole in the centre (Ø 6mm) to insert the shaft of the motor through it. The motor is fixed to this base tray, which is fixed to the box by screws. A second tray is Ø 210mm (3mm thick) with four small screws to fix the coupler motor connector. This tray is stuck together with the third tray using strong glue forming the middle tray (rotating tray). A third tray is Ø 210mm (3mm thick) with seven Ø 55mm holes, one in the middle and six for the plates to be placed. Finally, a fourth tray (cover) is Ø 260mm circle sheet (6mm thick) connected to the base tray with four long screws. It has Ø 55mm hole to allow one plate to be exposed to air at a time. The design considered using Ø 55mm instead of Ø 90mm plates to make it more portable.

6.3.6 The C program

Control is defined by a simple C code using the Arduino library with two simple functions: A setup function to set the device up and initialise the rotation parameters, and a loop function to perform the rotation and control the periods of exposure.

Begin

Set steps per revolution = 400;

Set the connected pins 8,9,10,11;

Setup()

{

Set speed = 10;

}

Loop()

{

Set delay;

Move N steps; //each step is 9°

}

End

6.4 Testing and Validation

6.4.1 Safety and verification testing

Initial safety testing was performed by the electronic services workshop (Faculty of Engineering, University of Leeds) to ensure the safety and robustness of the device.

The device can carry six plates; only one of them can be exposed to air at a time. It is essential to ensure that the rotation does not cause bias in the alignment of the plates and the hole above them. Since the stepper-motor moves in steps of 0.9° (400 steps/360°), the complete cycle of the AMPAS sampler consists of 67, 66, 67, 67, 66, 67 steps. The alignment was tested in the laboratory and found to be accurate with less than 0.37mm bias.

The timing of AMDS air exposure was tested using a stopwatch for periods of 10s, 60s, 600s, 3600s. The timing was accurate in all cases since it was controlled by a C-function that provides timing accuracy to the millisecond.

6.4.2 Preparation of culture broth, agar plates and suspension for nebulising.

Initial biological testing under a controlled chamber condition was carried out by nebulising a well-defined microbial suspension into the Leeds aerobiology chamber, described below. A laboratory strain of *staphylococcus aureus* (ATCC 6538) culture was prepared by transferring a loopful of bacteria into a 100ml of sterilised nutrient broth (Oxoid Ltd, UK). This culture broth was then incubated at 37°C for 48h.

TSA plates of Ø 90mm (Oxoid Ltd, UK) were prepared in the same way as in chapter 5. These plates were used for growing stock cultures, CFU counts and comparing between AMPAS and manual plate exposure in the real world settings (office and hospital pilot studies). The TSA plates of Ø 55mm used in AMPAS were prepared using pouring methods. The manufacturer's instructions (Oxoid Ltd, UK) were followed to prepare the agar for 500ml of the medium in duran bottles. The mixture was hand shaken to make sure it is completely mixed, Then, the agar was autoclaved at 121 °C for 15 minutes and later left to cool at 60 °C before pouring a 15ml into the Ø 55mm Petri dishes under aseptic conditions. Both the TSA plates of Ø 90mm and Ø 55mm agars were all left to cool and become solid, and then stored at room temperature to be used whenever required.

To find the concentration of the strain in the culture broth, it was diluted five folds (10^{-5} concentration) using serial dilutions with 9ml distilled water that was autoclaved at 121 °C for 15 minutes and left to cool before being used (Figure 6:4) 0.1 ml of the fifth bottle was pipetted and dispensed on the TSA, then incubated at 37°C for 24h for counting. The concentration of the strain in the culture broth was (~1.22 x10^{9} cfu/ml).

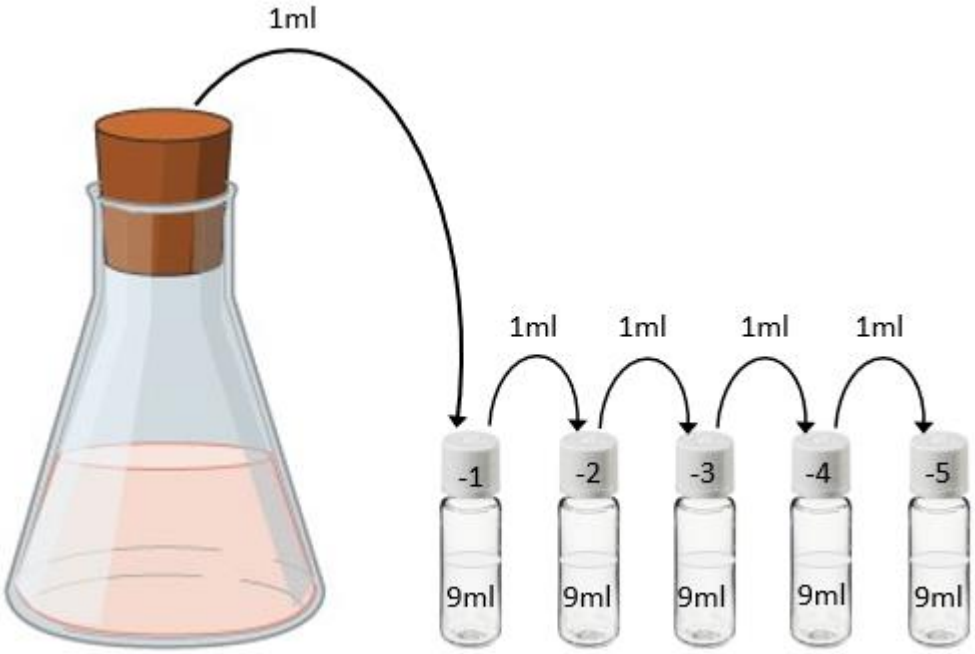

Figure 6:4 The serial dilution process using 9ml of distilled water in McCarthy bottles.

Aerosolisation was carried out using a 6-jet Collison nebuliser. The suspension fluid inside the Collison nebuliser vessel was created by adding a 1ml form the second McCarthy bottle sample of the serial dilutions. then adding it to 99 ml distilled water to achieve a concentration of (~1.22 $x10^5$ cfu/ml). The Collison 6-jet nebuliser (BGI, USA) operating at 12 $L.min^{-1}$, located outside the chamber, was used to generate the aerosolised *S. aureus* through the tube and into the centre of the chamber to produce aerosols in the range of 0.3-10 µm diameter (Figure 6:5).

Figure 6:5 The suspension fluid in the Collison nebuliser.

6.4.3 Air sampling

The bioaerosols were collected onto TSA using the 6-stage Anderson air sampler that was operated at a flow-rate of 28 $l.min^{-1}$ for four minutes. These six stages represent the lungs and allow different ranges of particles' size to go through (0.65, 1.1, 2.1, 3.3, 4.7 and 7 μm diameter)

The sampler was located externally to the chamber in the ante-room and air samples were taken using tubes via a sample port at the collection point at a height of 150 cm, positioned 30cm from the air outlet and 60cm from the adjacent wall. The location of the collection point has been shown previously to be representative of the average bioaerosol concentration of the whole chamber. Sample plates were cultured for 24 hours and counted, and positive hole correction factors were applied to the results as demonstrated in chapter 5.

6.4.4 Statistical analysis

For the statistics of this work, SPSS version 27 was used. Significance levels were considered < 0.05. The The one-way analysis of variance (ANOVA) test was performed to find the acceptable design of AMPAS in the chamber tests. An independent t-test was applied to compare the mean of

ACC load by using the AMPAS device with the mean of ACC using regular open plates over time in real-world tests.

6.4.5 Testing AMPAS design and exposure time in the chamber

The first test is to check that there will not be any bacteria spill over onto plates; the second test is to check that a consistent deposition occurs onto all the plates. For both tests, this should be under a controlled steady-state condition.

6.4.5.1 Configuring and adjusting the settings for the controlled aerobiological chamber

Experiments were conducted in the controlled aerobiology chamber at the University of Leeds, the dimensions used were similar to a single-bed room at the hospital (32.25 m^3): 4.26m (L) x 3.36m (W) x 2.26m (H). The mechanical air inlet is located towards the ceiling in one corner of the room, and the outlet is located diagonally opposite towards the floor (Figure 6:6).

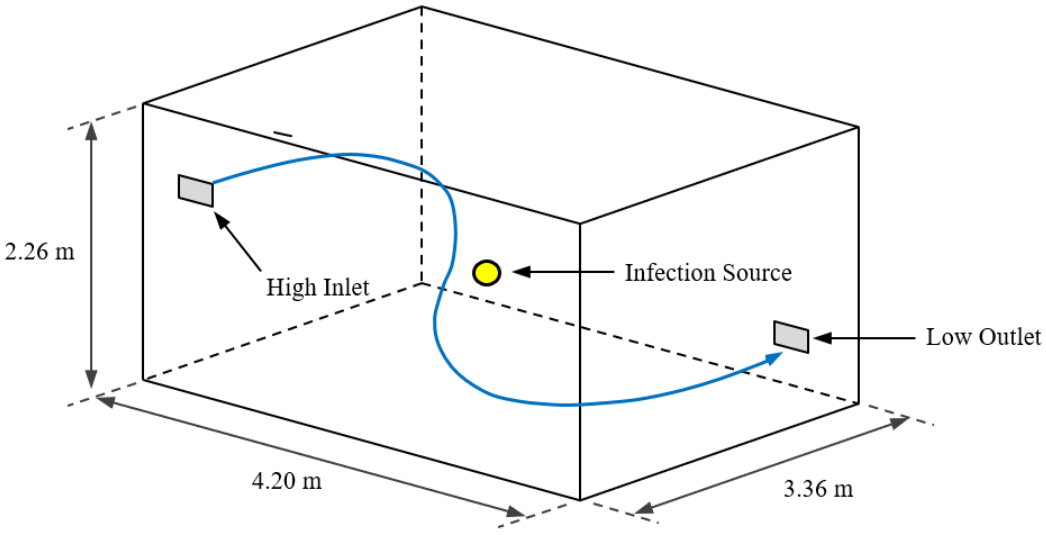

Figure 6:6 The aerobiological chamber dimensions and ventilation

The relationship between the ventilation rate and the number of air changes per hour (ACH) is illustrated in equation (6.1) (Atkinson et al., 2009).

$$N = \frac{Q \times 3600 \times 0.001}{V} \qquad (6.1)$$

N is air-change rate per hour (h^{-1}), Q is Ventilation rate ($l.s^{-1}$), V is room volume (m^3), 3600 to change units second to hour ($s.h^{-1}$) and 0.001 is change unit litre to cubic meter ($m.l^{-1}$).

To accurately set the ACH during experiments, a hand-held balometer (Digital Balometer TSI, Model PH721, TSI Incorporated, Shoreview, MN) was used to measure the flow at the inlet and outlet grilles in the chamber and the values of the corresponding fan settings were recorded. The fan settings were adjusted according to the acquired results as shown in Table 6:1, in order to make sure that a negative pressure within the chamber is maintained.

Table 6:1 Air change rate corresponding to the ventilation rate and the supply and extract fan settings**.**

Mechanical air located	Ventilation rate ($l.s^{-1}$)	Air Change rate per hour (h^{-1})*	Chamber system setting	
			Supply fan	Extractor fan
High inlet	25	2.79	9	
Low outlet	28	3.13		11.5
High inlet	53	5.92	16	
Low outlet	54	6.03		17
High inlet	99	11.05	32	
Low outlet	104	11.61		25

*This column was calculated based on equation 6.1.

6.4.5.2 Expected concentration and the role of deposition under the steady state condition

To be able to check the consistency of deposition rate onto the plates using AMPAS, the chamber must be under steady-state conditions. The chamber is designed to have an inlet with high-efficiency particulate absorbing (HEPA) filters for allowing clean air to be supplied into the chamber and for the outlet to safely extract air from the chamber. It is designed so that there is no recirculation of the extracted air, meaning that all microorganisms are generated from the source inside the chamber and that no microorganisms can enter through the clean air inlet. Thus, the rate of change in microbial concentration in the room air, assuming the air is well mixed, can be found using equation 6.2 (Beggs et al., 2006).

$$V\frac{dc}{dt} = q\beta - CV\lambda_T \qquad (6.2)$$

Where,

C is the bioaerosol concentration at time t (cfu.m^{-3}), V is the room volume (m^3), q is the constant microorganism's generation rate in the room space (cfu.s^{-1}), and β is the efficiency of generation rate. λ_T is the total loss rate (h^{-1}) which is the combination of the loss rate due to ventilation λ_v (N), loss rate due to deposition onto surfaces λ_d and loss rate due to decay γ. Assuming the loss rate due to deposition onto surfaces, loss rate due to decay, nebuliser efficiencies and sampler efficiencies were the same in all experiments, then the relative concentration in air is determined by ventilation rate only. By denoting the initial concentration of the room C_0, equation 6.2 can be solved to yield the concentration at time (t) as shown in equation 6.3

$$C = \frac{q}{\lambda_T V} + \left(C_0 - \frac{q}{\lambda_T V}\right) e^{-\lambda_T t} \qquad (6.3)$$

Here q was estimated as 444 cfu.s^{-1} (the concentration of suspension was 1.2 x 10^5 cfu/ml times the reduction rate 0.0037 ml. s^{-1}), N was 0.0017 s^{-1} (6/3600), V was 32.25 m^3 and C_0 was zero.

At the steady-state, there is no change in the concentration, meaning that the rate of change at the left-hand side of equation 6.2 is zero. By equating equation 6.2 to zero, the concentration at the steady-state can be calculated using equation (6.4) which indicates that the bioaerosol concentration should be inversely proportional to the total loss rate.

$$C = \frac{q}{\lambda_T V} \qquad (6.4)$$

According to equation (6.4), the steady-state condition will occur after one hour of continuous *staphylococcus aureus* generation to reach the maximum value of 6822 cfu.m^3, when assuming loss is due to ventilation only, i.e. no nebuliser loss, no deposition, no decay in the air in the room and no sampler losses. This value was higher than the results obtained from the pilot study result, as shown in (Figure 6:7).

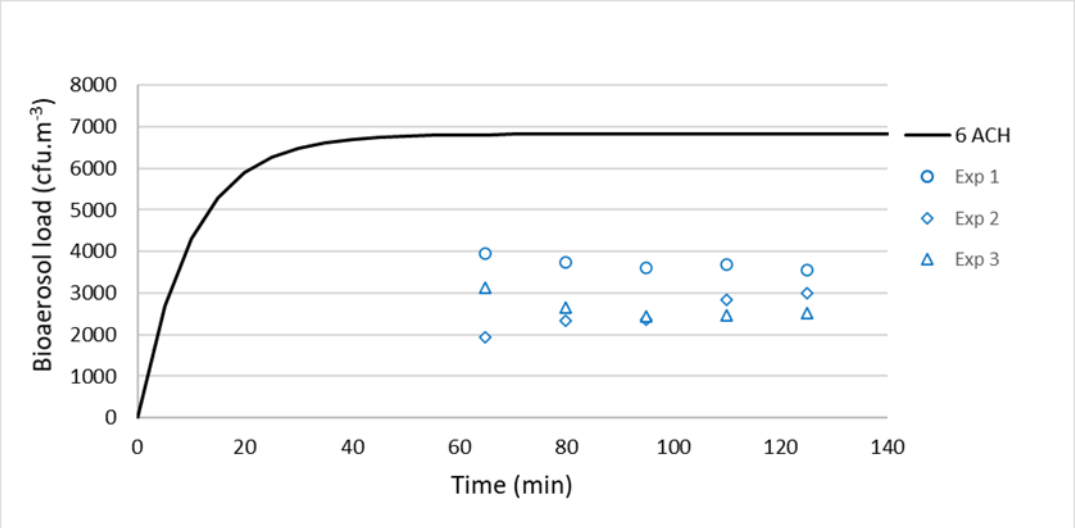

Figure 6:7: The bioaerosol load reaching the steady-state theoretically after one hour

The measured value is around half of the theoretical value, indicating that losses are significant and need to be accounted for. This difference can be due to not knowing the loss rate due to deposition on to surfaces, loss rate due to decay, nebuliser and sampler efficiencies. Thus. Equation (6.2) could not be used to determine the deposition loss rate while sampling the air over time because the generation rate of microorganisms inside the chamber was difficult to measure accurately since it depends on the efficiency of the nebuliser and sampling, which are unknown. The efficiency of the transmission path from the nebuliser to the chamber and the survival rate of microorganisms inside the nebuliser vessel is also unknown. This chapter demonstrates how the novel AMPAS device can be used to enable measuring the time series surface samples, which can be used to determine the loss rate of microorganisms due to deposition on surfaces inside the chamber.Under the steady-state conditions, as demonstrated in chapter 4 (equation 4.6 and 4.7), the total loss rate due to deposition on surfaces can be calculated as follows:

$$\lambda_d = \frac{C_{sf}\, A_f}{C\, V} + \frac{C_{sw}\, A_w}{C\, V} + \frac{C_{sc}\, A_c}{C\, V} \qquad (6),$$

where,

$C_{sf}, C_{sw}, and\ C_{sc}$ are the indoor deposited microorganisms' concentration on the floor, walls and ceiling, respectively ($cfu.m^{-2}.h^{-1}$). $A_f,\ A_w\ and\ A_c$ are the internal surface area of the floor, walls and ceiling, respectively (m^2).

6.4.5.3 Checking the negative control (no contamination spill onto covered plates)

In the design of AMPAS, it is assumed that when the test plate is exposed to air through the hole (positive control), the other five plates should be completely protected, and no microbial deposition should be detected on them as long as the device does not rotate (negative control). To test the negative control, a chamber experiment was conducted with inlet air temperature 26.5 ± 0.5 °C, relative humidity 37 % ± 1 %, operated at a constant ventilation rate 6 ACH under a small negative pressure and was sealed so no one can enter. Four AMPAS devices were put close together in front of the ventilation inlet and to the left of the nebuliser inlet where highest concentration is expected (Figure 6:8). The AMPAS devices were not operated in this experiment; plate 1 was exposed to air and the other plates 2-6 were covered throughout all the experiment.

A continuous release of aerosolised *S. aureus* was introduced to the chamber for 165 minutes at 6 ACH. The air sampling process was performed externally to the chamber via a tube, using an Anderson 6-stage impactor at a flow rate of 28L min^{-1}. Five samples were taken onto TSA agar plates for 4 minutes at 15-minute intervals, for three replicates for the experiment. The air sample collection point was placed at a 50 cm height, 20 cm from the air outlet, and 64 cm from the adjacent wall.

The results from the initial experiment showed that all plates had similar amounts of deposition of *S. aureus*, indicating that the microorganisms from the air are depositing on the plates even when they are supposed to be covered (Figure 6:9). This shows that deposition is complicated, with air movement through the sampler device enabling deposition onto plates that were not open vertically to the air. Following the experiment, the ventilation rate was changed to 12 ACH for 30 minutes for cleaning purposes before the operator entered the room.

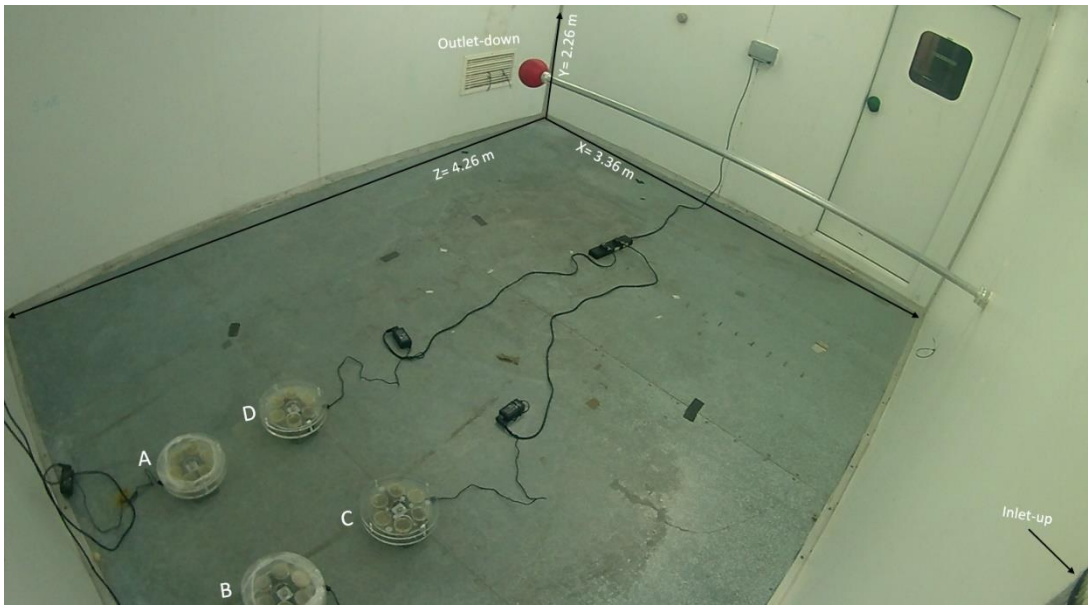

Figure 6:8 AMPAS placement in the controlled environment chamber.

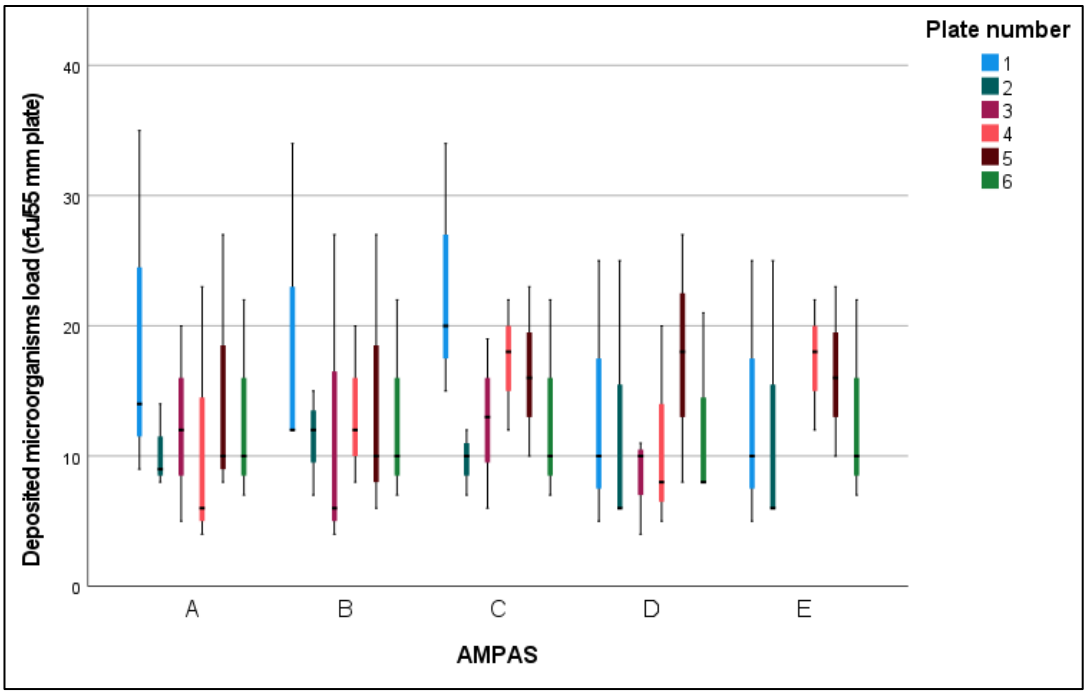

Figure 6:9: Sampling results from the negative control experiment before making improvements to the device.

It was concluded that bacteria could enter the hole through the gap between the top tray and the edge of the plate and travel to the other plates or through the perimeter of AMPAS (between the base and top trays). To solve

this problem, further tests were performed with some modifications. Plastic wrap was wrapped around the periphery of AMPAS for devices (A) and (B). The gap between the edge of plate and the top tray was reduced from 5mm to 1mm in devices (A) and (C), while device (D) was used as the original design without adjustments (5mm gap, no plastic wrap). A second set of tests were carried out to identify which design would provide higher protection to the covered plates; the experiment was replicated three times for validation.

The use of plastic wrap (A and B) had the most significant effect and together with reducing the gap to 1mm (A) significantly ($p < .0001$) improves the reliability of the collected data and eliminates the problem of undesired contamination (Figure 6:10).

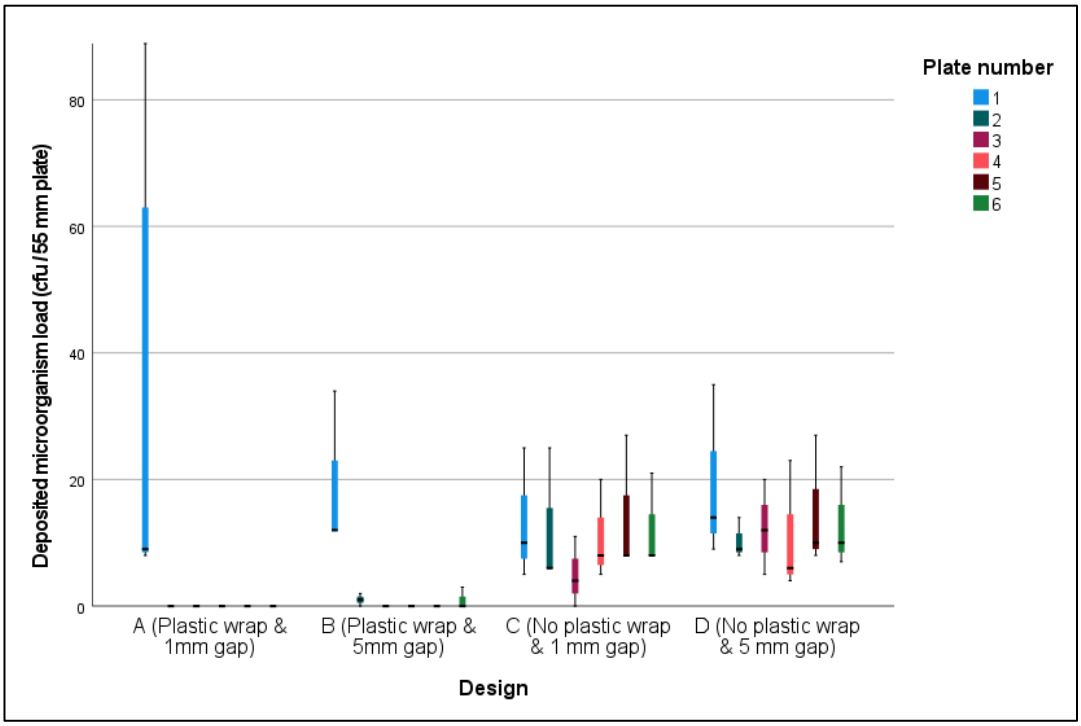

Figure 6:10 AMPAS negative control experiment in the chamber with four different designs A-D

6.4.5.4 Checking the consistency of deposition rate onto the plates using AMPAS

Experiments were carried out to determine whether the AMPAS device measured consistently on all of the sample plates. The four AMPAS devices were placed in the chamber in a similar placement to that of the previous

experiment. The plates were numbered from 1-6, where plate number 1 is under the hole at the beginning, plate number two is next to it. The rotation was clockwise from plate number one to plate number six. The device was programmed to wait for 60 minutes (exposing plate number 1 for 60 minutes to air), then rotate each 15 minutes (exposing plate number 2-6) then return to position number 1 and stop rotating (Figure 6:11). The air sampling process was performed externally to the chamber as in the previous experiment.

The first 60 minutes was used to allow the room to reach the steady-state condition. After that, the air sampling was performed using plates 2-6 for one minute in 15-minute cycles for 75 minutes (5 replicates/experiment). The surface sampling was performed for 15 minutes in 15-minute cycles for 75 minutes (5 replicates/experiment). Then, the plates were placed in an incubator at 37 °C for 24 hours for counting.

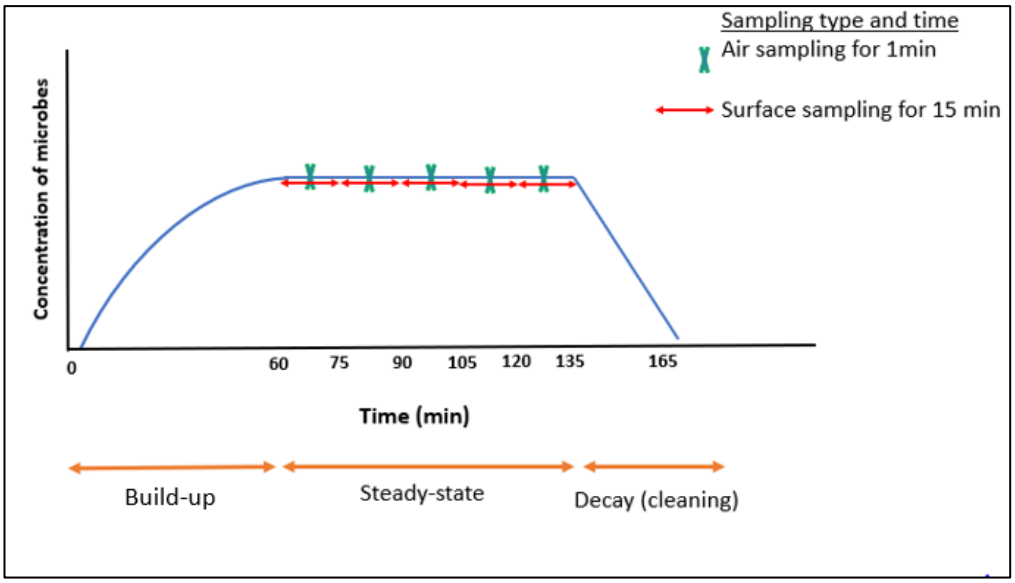

Figure 6:11: The concentration of microbes through the phases of AMPAS experiment (Build-up, Steady-state, and Decay).

As shown in Figure 6:12, the results were collated from all the samples across all four samplers and the average deposited microorganisms on plates 2-6 were found to be relatively similar, while plate number (1) has a higher deposition due to being exposed to air for a more extended period at the beginning and end of the experiment.

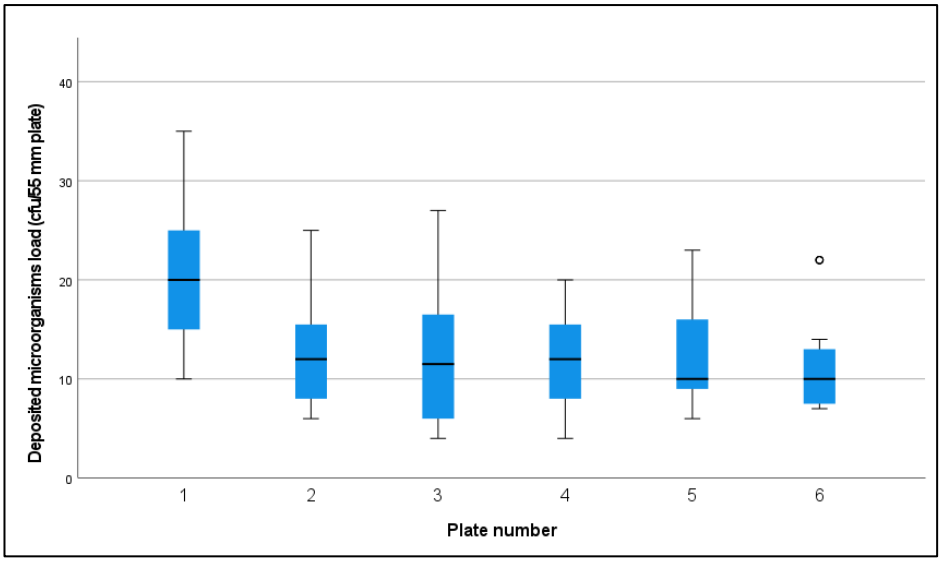

Figure 6:12: The deposited microorganisms on different plates during steady-state conditions.

Table 6:2 shows that the deposition on plate number (1) is significantly higher than the deposition on plates 2-6 according to the ANOVA test. There is no significant difference between plates 2-6, which means that the results are consistent across the plates.

Table 6:2: The difference between the deposited microorganisms on different plates

(I) Plate number	(J) Plate number	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	8.07*	2.406	0.017	0.98	15.16
	3	8.75*	2.552	0.014	1.23	16.27
	4	9.07*	2.406	0.005	1.98	16.16
	5	8.87*	2.406	0.006	1.78	15.96
	6	9.27*	2.406	0.004	2.18	16.36
2	1	-8.07*	2.406	0.017	-15.16	-0.98
	3	0.68	2.552	1	-6.84	8.2
	4	1	2.406	0.998	-6.09	8.09
	5	0.8	2.406	0.999	-6.29	7.89
	6	1.2	2.406	0.996	-5.89	8.29
3	1	-8.75*	2.552	0.014	-16.27	-1.23
	2	-0.68	2.552	1	-8.2	6.84
	4	0.32	2.552	1	-7.2	7.84
	5	0.12	2.552	1	-7.4	7.64
	6	0.52	2.552	1	-7	8.04

(I) Plate number	(J) Plate number	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
4	1	-9.07*	2.406	0.005	-16.16	-1.98
	2	-1	2.406	0.998	-8.09	6.09
	3	-0.32	2.552	1	-7.84	7.2
	5	-0.2	2.406	1	-7.29	6.89
	6	0.2	2.406	1	-6.89	7.29
5	1	-8.87*	2.406	0.006	-15.96	-1.78
	2	-0.8	2.406	0.999	-7.89	6.29
	3	-0.12	2.552	1	-7.64	7.4
	4	0.2	2.406	1	-6.89	7.29
	6	0.4	2.406	1	-6.69	7.49
6	1	-9.27*	2.406	0.004	-16.36	-2.18
	2	-1.2	2.406	0.996	-8.29	5.89
	3	-0.52	2.552	1	-8.04	7
	4	-0.2	2.406	1	-7.29	6.89
	5	-0.4	2.406	1	-7.49	6.69

* The mean difference is significant at the 0.05 level.

As shown in Figure 6:13, using five different devices with the final design (1mm gap and a plastic wrap on the perimeter) in the same settings yielded similar results for the average amount pooled for each device.

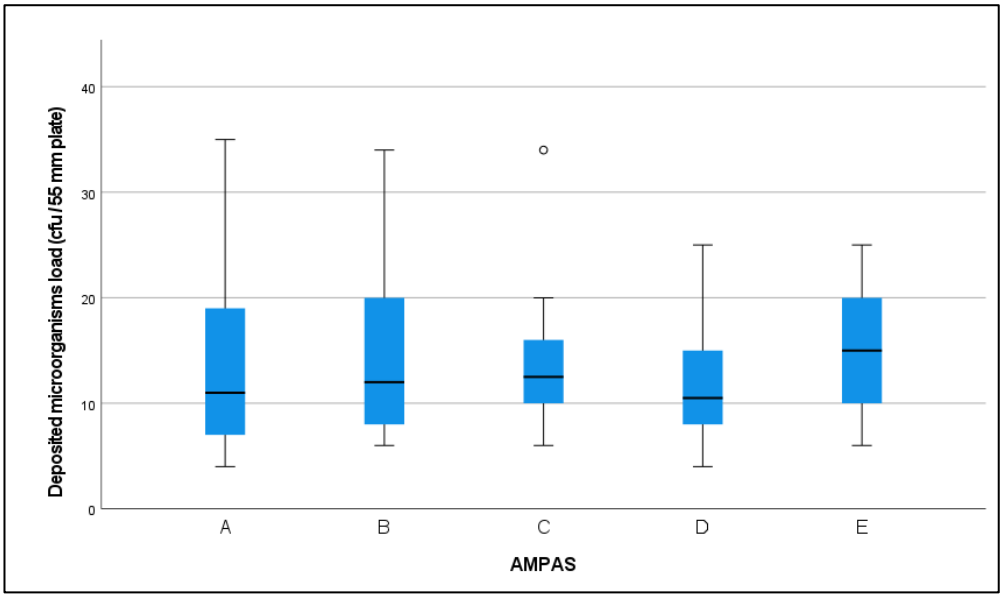

Figure 6:13 The deposited microorganisms on different devices.

Table 6:3 The difference between the deposited microorganisms on different devices.

(I) AMPAS number	(J) AMPAS number	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-0.94	2.197	0.993	-7.13	5.24
	C	-0.28	2.197	1	-6.46	5.91
	D	1.61	2.197	0.948	-4.57	7.8
	E	-1.7	2.304	0.947	-8.19	4.79
B	A	0.94	2.197	0.993	-5.24	7.13
	C	0.67	2.197	0.998	-5.52	6.85
	D	2.56	2.197	0.772	-3.63	8.74
	E	-0.76	2.304	0.997	-7.24	5.73
C	A	0.28	2.197	1	-5.91	6.46
	B	-0.67	2.197	0.998	-6.85	5.52
	D	1.89	2.197	0.91	-4.3	8.07
	E	-1.42	2.304	0.972	-7.91	5.06
D	A	-1.61	2.197	0.948	-7.8	4.57
	B	-2.56	2.197	0.772	-8.74	3.63
	C	-1.89	2.197	0.91	-8.07	4.3
	E	-3.31	2.304	0.607	-9.8	3.18
E	A	1.7	2.304	0.947	-4.79	8.19
	B	0.76	2.304	0.997	-5.73	7.24
	C	1.42	2.304	0.972	-5.06	7.91
	D	3.31	2.304	0.607	-3.18	9.8

Table 6:3 clearly shows that there is no significant difference between the deposition of microorganisms using different devices with the same design and settings. This confirms that using the AMPAS device provides consistent results.

6.4.6 Testing AMPAS in an office environment

The AMPAS device was used in an office environment to test its ability to detect the low concentration of microorganisms in an office compared with the controlled chamber, and to test the impact of using small agar plates 55mm compared with the 90 mm plates, which are used often for conventional passive sampling. Tests were performed in a postgraduate office of the School of Civil Engineering at the University of Leeds on 23/01/2020. Five devices were positioned in the office, as shown in Figure 6:14. The time of exposure was set to 1 hour for each of the plates (1-5), while plate number 6 was not covered and was used for negative control. There were two open 90mm petri dishes placed on top of the AMPAS device that were both represented by their mean value as plate number 7; these

two were placed to compare their total amount with the accumulation of the other five plates (1-5). To test the effect of using the AMPAS device and using commercial sterile Petri-dishes.

Active air sampling was performed every 30 minutes at 100 l/min for 5 minutes using a Microbio MB2 single-stage impactor bioaerosol sampler (Fred Parrett UK) to find the Aerobic colony count (ACC) in the room.

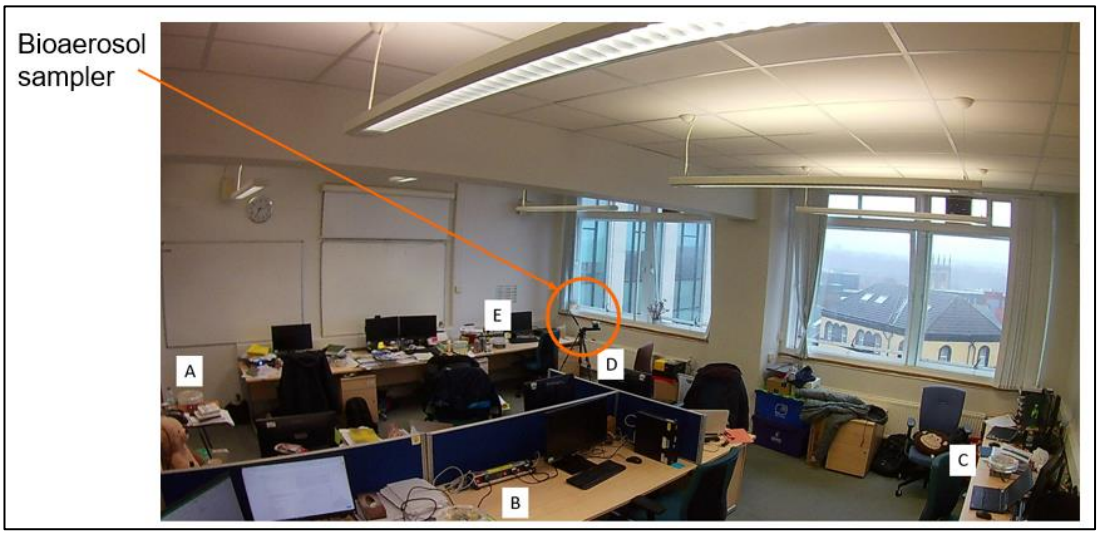

Figure 6:14 AMPAS placement in the postgraduate student's office

The ambient air temperature was 21.5 ± 0.5 °C, and relative humidity was 37 % ± 1 %. The concentration of airborne microbial concentration measured as ACC was (42.81 ± 13.89 cfu.m^{-3}). There is no significant difference ($p<0.05$) between the average number of ACC, which was collected on plate number 7 (on the top tray for 5 hours) and the total number of ACC, which was collected through the device at one-hour intervals (on plate number 1-5 for 5 hours).

For the five AMPAS devices placed in the postgraduate student's office, the concentrations collected are shown in Figure 6:15. In each case the data is from all five sequential samples taken with the AMPAS device. The results show that the deposition of microorganisms on surfaces was detected with varying concentrations due to being affected by the presence of human sources and the air ventilation by the window and the door. It is much easier and more time-efficient to use AMPAS since it does not require manual handling for each interval. It is also more accurate to use AMPAS because it minimises the interference with humans and give more reliable readings.

This automation allows tests to run in a flexible way and overcomes several constraints that prevent open Plate tests including time, number of staff required, emergencies.

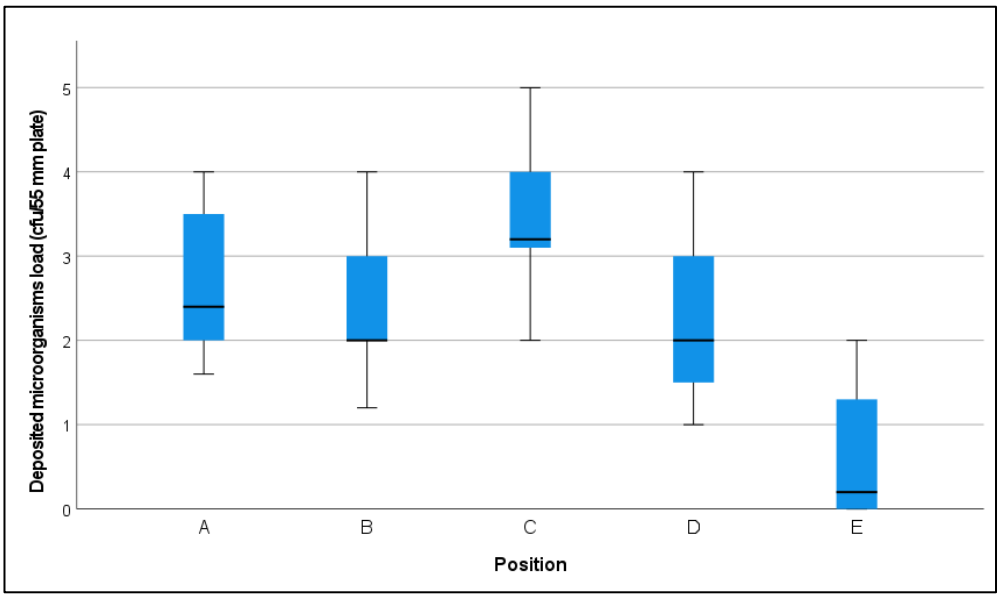

Figure 6:15 Concentrations of ACC using AMPAS in different locations of the postgraduate students' office.

6.4.7 Testing AMPAS in the hospital

The hospital environment imposes significant challenges since it is occupied by patients, some of which may have contagious diseases making it difficult to researchers to perform sampling for safety reasons. The use of the AMPAS device in such environments can be particularly beneficial as it solves several problems and minimises the human interaction required. To test the efficiency of the AMPAS device in capturing the deposited microorganisms, a comparison between sampling with Ø 55 mm and Ø 90 mm plates, similar to the office study, was performed.

The pilot study was performed in the respiratory ward in St. James's hospital on 10/03/2020, as shown in Chapter 5 (5.2.3). The time of exposure was set to 30 minutes for each of the plates (1-6). As recommended by (Pasquarella et al., 2000), AMPAS and conventional petri-dishes are placed at a 1m height and 1.5m away from the window. The plates were each exposed for 30-minute intervals over a total time from 8:00am – 4:00pm.

The result shows that samples using Ø 90 mm open Petri dishes yielded a deposition rate of 0.14 ± 0.06 (0.06 – 0.25) cfu.cm^{-2}.30 minutes^{-1}. Using the AMPAS device with Ø 55 mm plates yielded a deposition rate of 0.16 ± 0.11 (0.05 – 0.28) cfu.cm^{-2}.30 minuties^{-1}. There is no significant difference in ACC deposition rate using AMPAS or 90 mm open Petri dishes plates ($p<0.05$).

6.5 Summary

This chapter has introduced a novel automated multiplate passive air sampler (AMPAS) device that can be programmed and configured to obtain passive air samples in a flexible way tailored to the investigator's needs. The device can autonomously cover or expose up to 5 plates to the air for a configurable period of time in the chamber setting and up to 6 plates in a real environment. The device was tested in a lab for safety and robustness. The alignment of the plates was found to be accurate with a bias less than 0.37mm. The timing was controlled by a C-program and was accurate to the millisecond.

The reliability of the device was tested in the controlled chamber to find whether there is a spill over onto the covered plates and to check that the results obtained by different plates are consistent. The design and implementation of the device were altered and improved based on these results and the final design reduced the gap between the plates and the cover to 1mm and added a plastic wrap around the perimeter significantly improving the reliability of the device ($p<0.0001$) and eliminating the problem of undesired contamination. There was no significant difference in the results obtained from different devices nor in the results obtained from different plates on the same device, confirming that the results are consistent across plates and devices of the same configuration and settings.

The AMPAS device was able to collect samples in areas with lower concentrations of microorganisms in the postgraduate student's office, and there was no significant difference ($p<0.05$) between the results obtained using the AMPAS device with Ø 55 mm plates and Ø 90 mm Petri dishes in a hospital environment. Thus, AMPAS device can be used in a controlled environment and in the real-world environment.

7 The effect of ventilation rate on the deposition rate of microorganisms in a controlled microbiological chamber

7.1 Introduction

As demonstrated in chapter 4, there is evidence from the literature that microorganisms can be deposited on surfaces but that the effect of the ventilation rate on the deposition rate is not yet fully understood. Chapter 5 and chapter 6 supports this evidence by showing real-world sampling results in a hospital environment and an office setting, confirming that there is a gap in the knowledge and a fluctuation of the deposition rate measured in the hospital.

This chapter investigates the effect of ventilation and spatial location on the loss rate due to the deposition of microorganisms on surfaces. Experiments were carried out in a controlled chamber setting using a nebulised suspension of a test microorganism, and samples were taken in the air and on surfaces simultaneously during steady state conditions. The AMPAS device presented in chapter 6 offers excellent features to enable time series surface sampling. The effect of different dry particle sizes on the deposition rate of bioaerosols on surfaces was not investigated due to the limited time and the restrictions imposed on the access to the lab and university resources caused by the Covid-19 pandemic.

7.2 Methodology

The experiments in this chapter were conducted in the controlled aerobiological chamber described in chapter 6 using an automated multiplate passive air sampler (AMPAS) and an Anderson 6-stage impactor. The preparation, generation and sampling of *Staphylococcus aureus* are all as described in chapter 6 (6.4.2). There were six experiments at the ventilation rate of 3 ACH, and six experiments at the ventilation rate of 6 ACH. At each ventilation rate, three experiments were performed with samples taken near the outlet and three near the inlet with five replicates at different time intervals at the steady-state conditions for each experiment giving a total of 60 values.

The air sampling was performed outside the chamber through a tube, using an Anderson 6-stage impactor at 28L min^{-1} flow rate. The TSA agar plates were placed at stages number 5 and 6 only and the air was sampled five times for 4 minutes at an interval of 15-minute. The bioaerosol air sample collection point was at a height of 150cm, either positioned 30cm from the air outlet and 60cm from the adjacent wall or positioned 30cm from the air inlet and 60cm from the adjacent wall.

The surface sampling was performed using five AMPAS devices (as demonstrated in chapter 6) for 15 minutes in 15-minute cycles five times reporting the average value on the floor surface for the five AMPAS devices at each time interval. The AMPAS devices were positioned close to each other either at a collection point 50 cm from the air outlet and 60cm from the adjacent wall, or positioned at a collection point 50cm from the air inlet and 66 cm from the adjacent wall. The air and surface sample locations are shown in Figure 7:1.

The first 60 minutes of each experiment was used to allow the room to reach the steady-state conditions as demonstrated in chapter 6 (6.4.5.2). After that, the air and surface sampling was performed for 75 minutes. Then the ventilation rate was set to 12 ACH for 30 minutes for cleaning. Following the experiment the plates were placed for a full day in an incubator at 37 °C for counting. The ambient air temperature and relative humidity were 22.5 ± 0.5 °C, and 49 % ± 1 %, respectively.

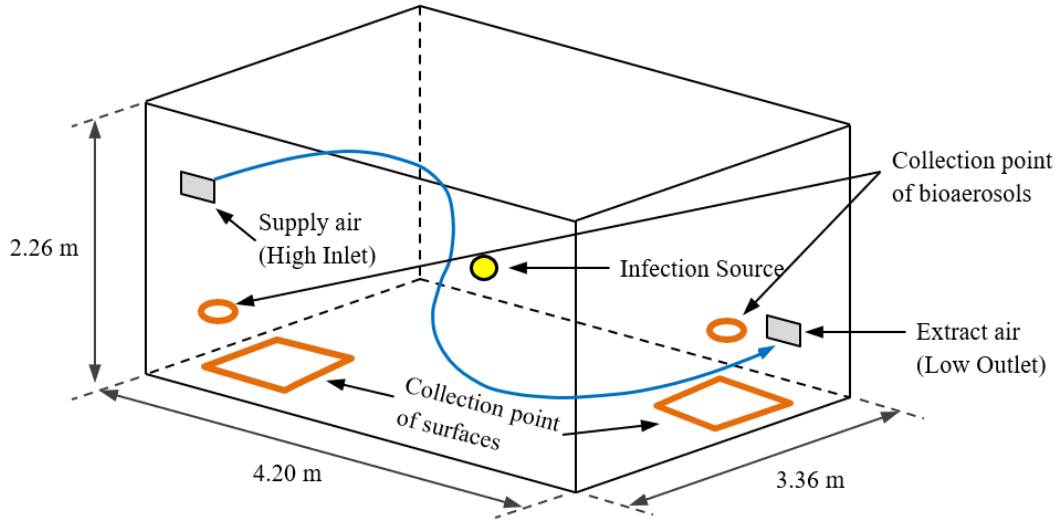

Figure 7:1 The aerobiological chamber dimensions and the collection points of air and surfaces.

The percentage of bioaerosols load reduction and deposition microorganism rate through the effect of changing ventilation from 3 to 6 ACH was calculated using equation 7.1

$$R_l = \frac{\sum_1^i \left(\frac{m_{3,l} - n_{i,6,l}}{m_{3,l}}\right)}{i} \times 100\% \qquad (7.1)$$

Where,

R_l is the percentage of reduction in a specific location (inlet or outlet), i is the number of samples of data, $m_{3,l}$ is the mean of bioaerosols load of deposited microorganism load in specific location (inlet or outlet), $n_{i,6,l}$ is the single data value at the same location l for sample i.

7.3 Results and discussion

Table 7:1 shows the descriptive statistics of bioaerosols load in air and deposition rate of microorganisms on surfaces under steady-state conditions across all the experiments. Since, as expected, the experiments at a ventilation rate of 6 ACH resulted in a reduction in the airborne microorganism concentration, the reduction was calculated as a percentage to show the difference in concentration from experiments at a ventilation rate of 3 ACH.

At a ventilation rate of 3 ACH, the measured bioaerosols load near the inlet and the outlet were 3797 ± 426 cfu.m^{-3} (2878 - 4437 cfu.m^{-3}) and 5599 ± 565 cfu.m^{-3} (4376 - 6767 cfu.m^{-3}), respectively. At 6 ACH under the same experimental conditions, the bioaerosols load near the inlet and the outlet were lower at 2218 ± 350 cfu.m^{-3} (1669 - 2861 cfu.m^{-3}) and 3167 ± 580 cfu.m^{-3} (1933 - 4571 cfu.m^{-3}), respectively. At 6 ACH ventilation rate, there was a lower airborne microorganism concentration, with 43% and 45% of the concentration measured at 3ACH for the collection points near the inlet and near the outlet, respectively. This result falls within the 50% reduction expectation, and the spatial variation will be discussed further below. The comparison between the values measured at the inlet and the values at the outlet were based on different experiments (different positioning of AMPAS) at similar conditions.

Table 7:1 Descriptive statistics of bioaerosol concentration and deposition rate of microorganisms on surfaces.

ACH	Air sampling collection point	Bioaerosols load (cfu.m^{-3})		Deposited microorganism load (cfu.m^{-2}.h^{-1})	
		Mean ± SD (Min-Max), n=sample size	Reduction percentage	Mean ± SD (Min-Max)	Reduction percentage
3	Near supply air (Inlet)	3797± 426 (2878 - 4437), n=15		3696 ± 1885 (758 - 7036), n=15	
	Near extract air (Outlet)	5599 ± 565 (4376 - 6767), n=15		9450 ± 4469 (4363 - 22171), n=15	
	Total	4698 ± 1035 (2878 - 6767), n=30		6573 ± 4470 (758 - 22171), n=30	
6	Near supply air (Inlet)	2218 ± 350 (1669 - 2861), n=15	43% ± 8%	2442 ± 910 (505 - 4042), n=15	33% ± 25%
	Near extract air (Outlet)	3167 ± 580 (1933 - 4571), n=15	45% ± 10%	5086 ± 2961 (1011 - 10611), n=15	44% ± 32%
	Total	2693 ± 674 (1669 - 4571), n=30		3764 ± 2548 (505 - 10611), n=30	

7.3.1 Bioaerosols load in air

The concentration in the air was approximately constant throughout each experiment and the samples taken between 65 and 125 minutes can be confidently considered at the steady state. There was no significant difference between the bioaerosols load collected after one hour for the five intervals period in the exact location under the same air change rate. This means that for both 3 and 6 air changes per hour, the chamber reached the steady-state conditions after one hour. Table 7:2 shows that there is no significant difference between the bioaerosols load with a ventilation rate of 3 ACH. The values for the collection point near the inlet and near the outlet were considered.

Figure 7:2 shows that although there is variability, the mean bioaerosol load in air does not change significantly, near the inlet nor near the outlet over the course of each experiment. This confirms that the chamber has reached steady-state conditions after 60 minutes.

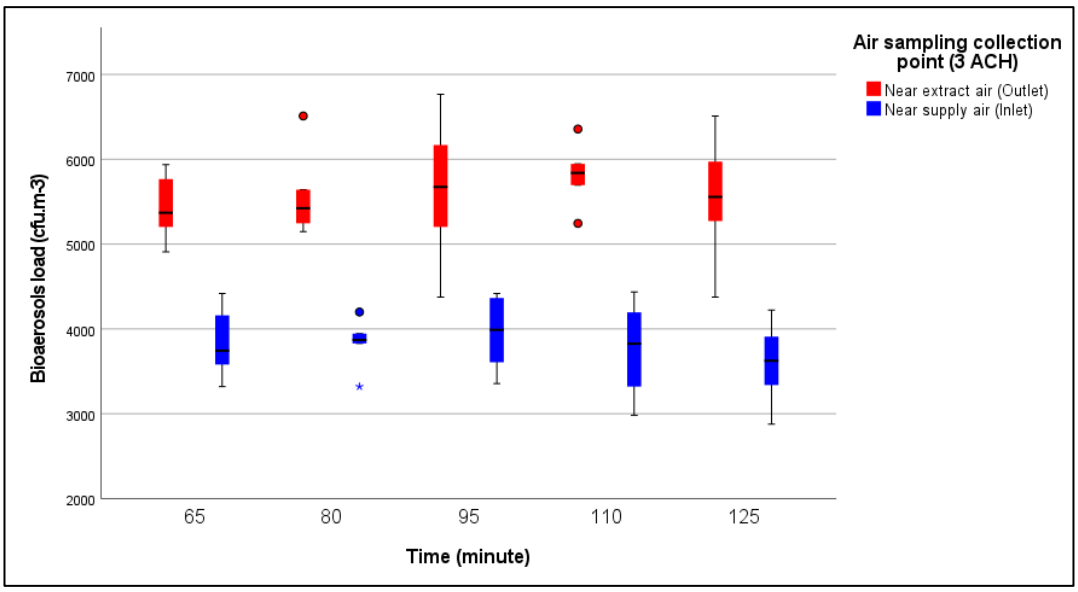

Figure 7:2: The bioaerosol load with time under steady-state conditions at 3 ACH near the inlet and the outlet

Table 7:2: Bioaerosol load at 3 ACH under steady-state conditions at different times

Bioaerosol collection point	(I) Time (minute)	(J) Time (minute)	Bioaerosols load ($cfu.m^{-3}$) Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Near the inlet	65	80	-11	254	1.000	-758	736
		95	-127	254	0.987	-874	620
		110	64	254	0.999	-683	811
		125	229	254	0.895	-518	975
	80	65	11	254	1.000	-736	758
		95	-116	254	0.991	-862	631
		110	75	254	0.998	-672	822
		125	240	254	0.877	-507	987
	95	65	127	254	0.987	-620	874
		80	116	254	0.991	-631	862
		110	190	254	0.943	-557	937
		125	355	254	0.635	-392	1102
	110	65	-64	254	0.999	-811	683
		80	-75	254	0.998	-822	672
		95	-190	254	0.943	-937	557
		125	165	254	0.965	-582	912
	125	65	-229	254	0.895	-975	518
		80	-240	254	0.877	-987	507
		95	-355	254	0.635	-1102	392
		110	-165	254	0.965	-912	582
Near the outlet	65	80	-139	342	0.994	-1143	864
		95	-218	342	0.967	-1221	786
		110	-395	342	0.776	-1398	609
		125	-115	342	0.997	-1119	889
	80	65	139	342	0.994	-864	1143
		95	-78	342	0.999	-1082	925
		110	-255	342	0.943	-1259	749
		125	24	342	1.000	-979	1028
	95	65	218	342	0.967	-786	1221
		80	78	342	0.999	-925	1082
		110	-177	342	0.985	-1181	827
		125	103	342	0.998	-901	1106
	110	65	395	342	0.776	-609	1398
		80	255	342	0.943	-749	1259
		95	177	342	0.985	-827	1181
		125	280	342	0.923	-724	1283
	125	65	115	342	0.997	-889	1119
		80	-24	342	1.000	-1028	979
		95	-103	342	0.998	-1106	901
		110	-280	342	0.923	-1283	724

Table 7:3: Bioaerosol load at 6 ACH under steady-state conditions at different times

Bioaerosol collection point	(I) Time (minute)	(J) Time (minute)	Bioaerosols load ($cfu.m^{-3}$) Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Near the inlet	65	80	104	214	0.988	-524	733
		95	-32	214	1.000	-661	596
		110	116	214	0.982	-512	745
		125	-18	214	1.000	-646	611
	80	65	-104	214	0.988	-733	524
		95	-137	214	0.967	-765	492
		110	12	214	1.000	-617	640
		125	-122	214	0.978	-751	506
	95	65	32	214	1.000	-596	661
		80	137	214	0.967	-492	765
		110	148	214	0.956	-480	777
		125	14	214	1.000	-614	643
	110	65	-116	214	0.982	-745	512
		80	-12	214	1.000	-640	617
		95	-148	214	0.956	-777	480
		125	-134	214	0.969	-762	494
	125	65	18	214	1.000	-611	646
		80	122	214	0.978	-506	751
		95	-14	214	1.000	-643	614
		110	134	214	0.969	-494	762
Near the outlet	65	80	104	354	0.998	-935	1142
		95	232	354	0.964	-806	1271
		110	15	354	1.000	-1024	1053
		125	276	354	0.934	-762	1315
	80	65	-104	354	0.998	-1142	935
		95	129	354	0.996	-910	1167
		110	-89	354	0.999	-1128	949
		125	173	354	0.988	-866	1211
	95	65	-232	354	0.964	-1271	806
		80	-129	354	0.996	-1167	910
		110	-218	354	0.971	-1256	821
		125	44	354	1.000	-994	1082
	110	65	-15	354	1.000	-1053	1024
		80	89	354	0.999	-949	1128
		95	218	354	0.971	-821	1256
		125	262	354	0.945	-777	1300
	125	65	-276	354	0.934	-1315	762
		80	-173	354	0.988	-1211	866
		95	-44	354	1.000	-1082	994
		110	-262	354	0.945	-1300	777

Table 7:3 and Figure 7:3 show that at the 6 ACH ventilation rate, there is still no significant difference in the bioaerosol load in air between samples taken at 65 minutes and 125 minutes. This also applies to both collection points near the inlet and the outlet.

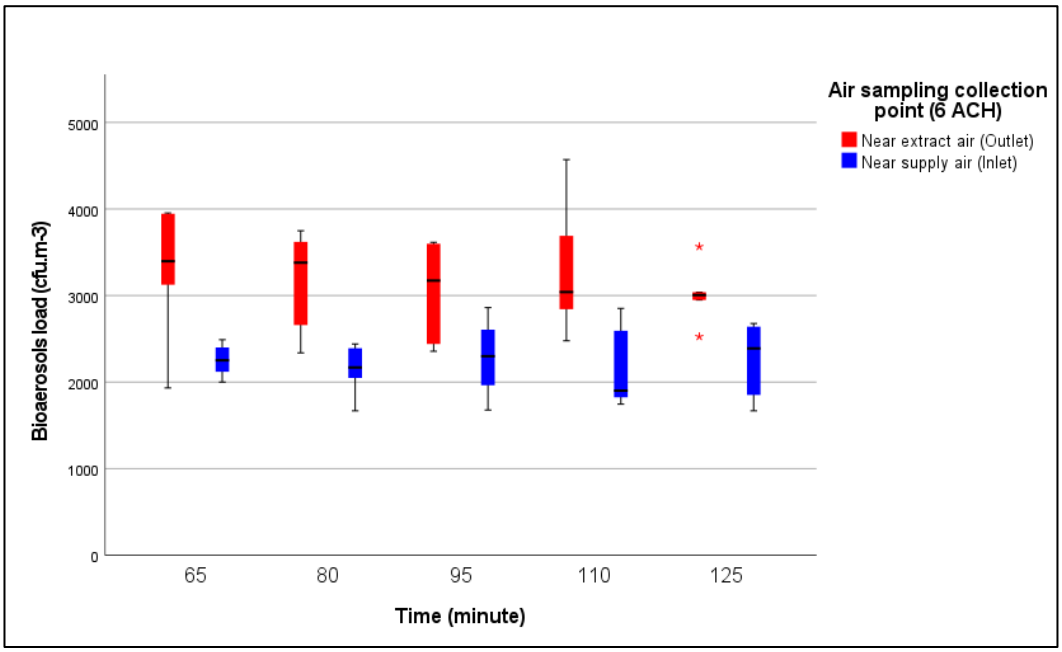

Figure 7:3: The bioaerosol load at 6 ACH near the inlet and the outlet at different times

Figure 7:4 pools the data from all five samples in each of the three experiments at each condition. It shows that the bioaerosol load near the extract air (Outlet) was significantly higher ($p < 0.001$) than bioaerosol load near the supply air (Inlet) at both 3 and 6 ACH ventilation rates. It also shows that the variability in aerosol concentration at different positions even in a reasonably well mixed room could be comparable to the difference that results from doubling the ventilation rate. This makes sense as fresh air supply affects the concentration of airborne microorganisms, especially near the inlet air collection point in these experiments. This observation highlights the need to consider a more efficient ventilation techniques and regime.

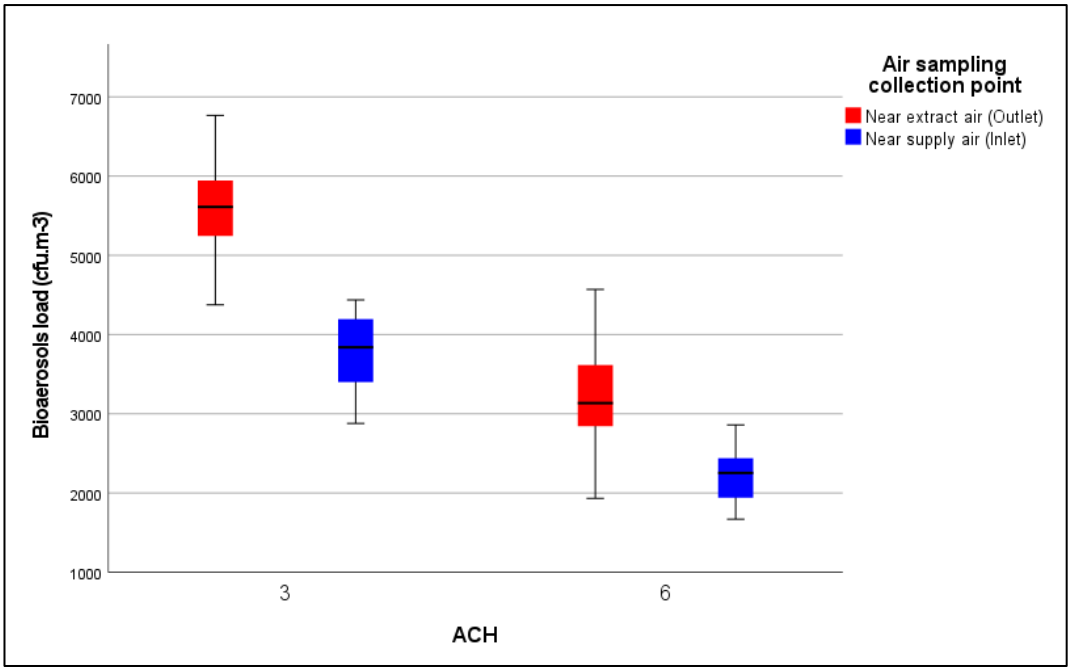

Figure 7:4: The bioaerosol load under the steady-state at 3 and 6 ACH ventilation rate

Using equation 7.1, the percentage of reduction in bioaerosols load when using ventilation at 6 ACH compared to using 3 ACH was found to be 43% ± 8% near the inlet and 45% ± 10% near the outlet (Table 7:1).

7.3.2 Deposited microorganism load

The deposited microorganism load under the steady-state conditions was found to have no significant difference across the 5-time intervals of collection at a ventilation rate of 3 ACH (Table 7:4 and Figure 7:5). The difference between the 5 AMPAS samples was shown to be not significant. The sample area of each plate is small 23.75 cm^2 so we cumulated the results to have more accurate results that cover a larger surface area.

Table 7:4: Deposited microorganism load at 3 ACH under the steady-state conditions at different times

Bioaerosol collection point	(I) Time (minute)	(J) Time (minute)	Bioaerosols load (cfu. 55 mm plate^{-1}.h^{-1}) Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Near the inlet	65	80	1.3	3	0.985	-6	9
		95	-2.5	3	0.868	-10	5
		110	-3.3	3	0.701	-11	4
		125	-0.8	3	0.998	-8	7
	80	65	-1.3	3	0.985	-9	6
		95	-3.8	3	0.584	-11	4
		110	-4.7	3	0.395	-12	3
		125	-2.2	3	0.917	-10	5
	95	65	2.5	3	0.868	-5	10
		80	3.8	3	0.584	-4	11
		110	-0.8	3	0.998	-8	7
		125	1.7	3	0.966	-6	9
	110	65	3.3	3	0.701	-4	11
		80	4.7	3	0.395	-3	12
		95	0.8	3	0.998	-7	8
		125	2.5	3	0.868	-5	10
	125	65	0.8	3	0.998	-7	8
		80	2.2	3	0.917	-5	10
		95	-1.7	3	0.966	-9	6
		110	-2.5	3	0.868	-10	5
Near the outlet	65	80	0.3	6	1.000	-18	19
		95	-3.7	6	0.976	-22	15
		110	4.5	6	0.950	-14	23
		125	5.7	6	0.891	-13	24
	80	65	-0.3	6	1.000	-19	18
		95	-4.0	6	0.967	-22	14
		110	4.2	6	0.962	-14	23
		125	5.3	6	0.911	-13	24
	95	65	3.7	6	0.976	-15	22
		80	4.0	6	0.967	-14	22
		110	8.2	6	0.689	-10	27
		125	9.3	6	0.575	-9	28
	110	65	-4.5	6	0.950	-23	14
		80	-4.2	6	0.962	-23	14
		95	-8.2	6	0.689	-27	10
		125	1.2	6	1.000	-17	20
	125	65	-5.7	6	0.891	-24	13
		80	-5.3	6	0.911	-24	13
		95	-9.3	6	0.575	-28	9
		110	-1.2	6	1.000	-20	17

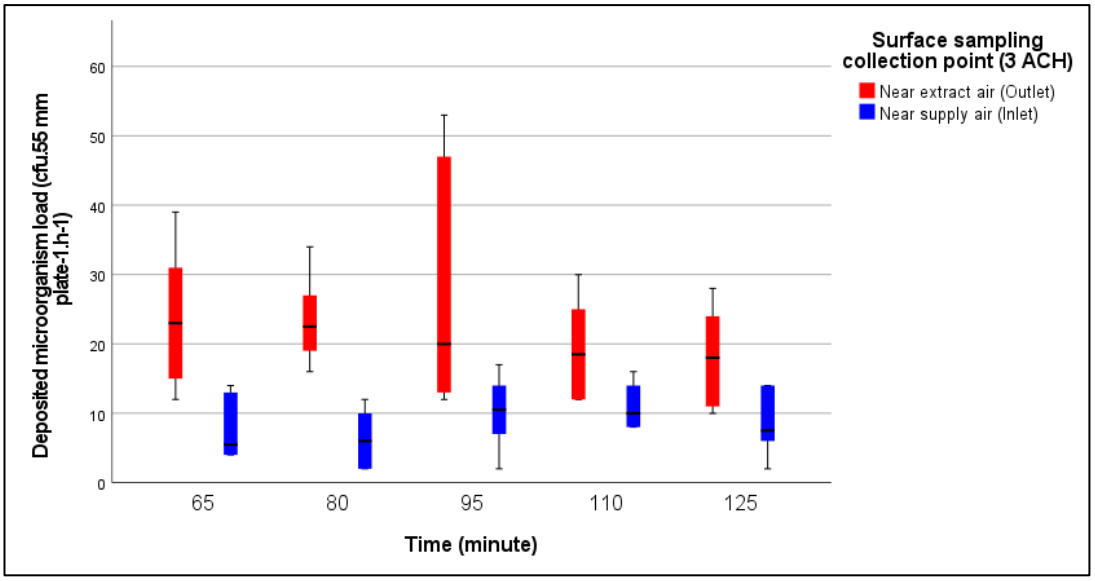

Figure 7:5: Deposited microorganism load at 3 ACH under the steady-state conditions at different times near the inlet and the outlet.

Table 7:5 and Figure 7:6 show that the mean deposited microorganism load under the steady-state conditions was also found to have no significant difference across the different time intervals of collection at a ventilation rate of 6 ACH.

As shown in Figure 7:7, there is more variation in the deposition samples near the outlet than the inlet, and they are also higher in both cases. This agrees with the literature and may be because there is a higher concentration of microorganisms near the outlet (Lai et al., 2012). The higher variation in the deposited microorganisms near the outlet is likely to be due to the positioning of the collection points. It can be clearly seen in Figure 7:1 that the extract air outlet is positioned lower than the fresh air inlet and closer to the collection point. This means that the airflow near the outlet is more disturbed than near the inlet, which may cause a higher variation in the results.

Table 7:5: Deposited microorganism load at 6 ACH under the steady-state conditions at different times

Bioaerosol collection point	(I) Time (minute)	(J) Time (minute)	Bioaerosols load (cfu. 55 mm plate^{-1}.h^{-1})	Std. Error	Sig.	95% Confidence Interval	
			Mean Difference (I-J)			Lower Bound	Upper Bound
Near the inlet	65	80	1.5	1	0.688	-2	5
		95	-0.2	1	1.000	-4	3
		110	0.0	1	1.000	-3	3
		125	2.5	1	0.219	-1	6
	80	65	-1.5	1	0.688	-5	2
		95	-1.7	1	0.599	-5	2
		110	-1.5	1	0.688	-5	2
		125	1.0	1	0.904	-2	4
	95	65	0.2	1	1.000	-3	4
		80	1.7	1	0.599	-2	5
		110	0.2	1	1.000	-3	4
		125	2.7	1	0.169	-1	6
	110	65	0.0	1	1.000	-3	3
		80	1.5	1	0.688	-2	5
		95	-0.2	1	1.000	-4	3
		125	2.5	1	0.219	-1	6
	125	65	-2.5	1	0.219	-6	1
		80	-1.0	1	0.904	-4	2
		95	-2.7	1	0.169	-6	1
		110	-2.5	1	0.219	-6	1
Near the outlet	65	80	-0.3	4	1.000	-13	12
		95	1.2	4	0.999	-12	14
		110	0.5	4	1.000	-12	13
		125	-0.2	4	1.000	-13	13
	80	65	0.3	4	1.000	-12	13
		95	1.5	4	0.997	-11	14
		110	0.8	4	1.000	-12	14
		125	0.2	4	1.000	-13	13
	95	65	-1.2	4	0.999	-14	12
		80	-1.5	4	0.997	-14	11
		110	-0.7	4	1.000	-13	12
		125	-1.3	4	0.998	-14	11
	110	65	-0.5	4	1.000	-13	12
		80	-0.8	4	1.000	-14	12
		95	0.7	4	1.000	-12	13
		125	-0.7	4	1.000	-13	12
	125	65	0.2	4	1.000	-13	13
		80	-0.2	4	1.000	-13	13
		95	1.3	4	0.998	-11	14
		110	0.7	4	1.000	-12	13

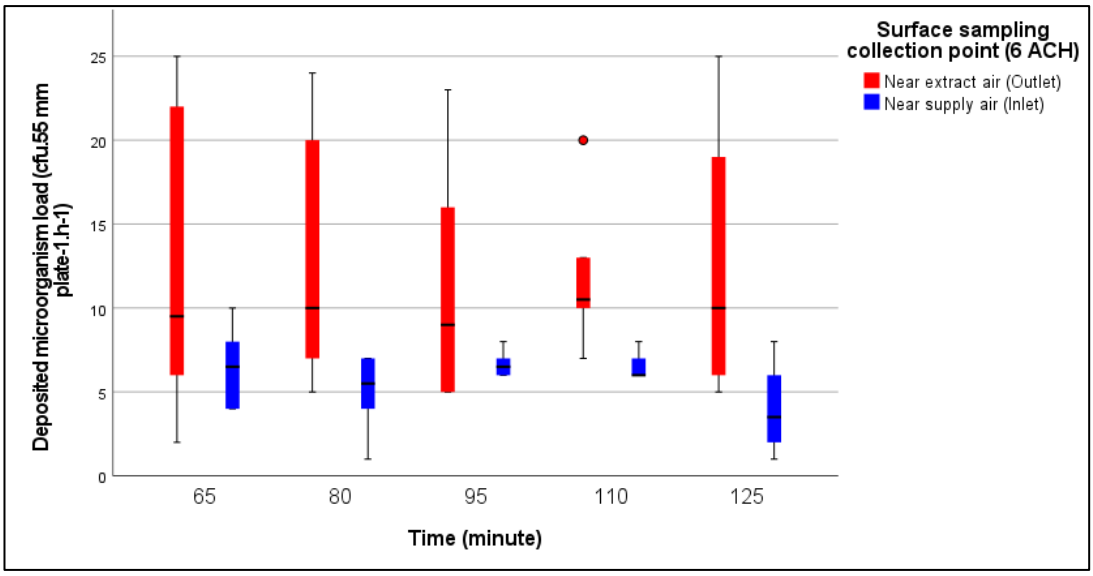

Figure 7:6: Deposited microorganism load at 6 ACH under the steady-state conditions at different times near the inlet and the outlet

The same variation is seen in Figure 7:7, which shows that the deposited microorganisms load near extract air was 9450 ± 4469 cfu.m^{-2}.h^{-1}, which is significantly higher ($p < 0.001$) than the deposited load near the inlet 3696 ± 1885 cfu.m^{-2}.h^{-1} at 3 ACH ventilation rate. This means that, on average, the deposited microorganisms are two and a half times higher near the outlet than near the inlet.

Furthermore, at a ventilation rate of 6 ACH, the deposited microorganism load near extract air was 5086 ± 2961 cfu.m^{-2}.h^{-1}, which is significantly higher ($p < 0.001$) than the deposited load near the inlet 2442 ± 910 cfu.m^{-2}.h^{-1}. This means that the deposited microorganisms are twice as high near the outlet compared to near the inlet.

A previous work (Lai et al., 2012), which was conducted in a Class II biological safety cabinet of size 0.650 m (L), 0.380 m (W), 0.284 m (H) and nebulised *Staphylococcus aureus,* has found that the deposition rate near the outlet was 1.53 and 1.79 times higher than near the inlet at a ventilation rate of 1.7 h^{-1} and 10.3 h^{-1} respectively.

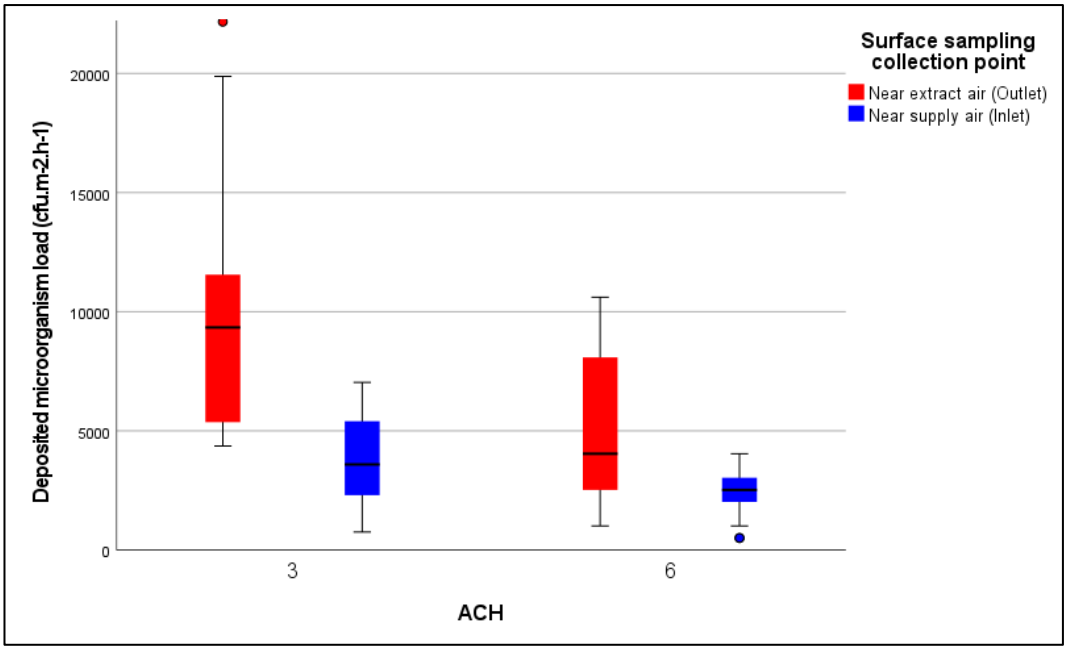

Figure 7:7: The mean deposited microorganisms load under the steady-state conditions at 3 and 6 ACH ventilation rates near the inlet and the outlet.

Using equation 7.1, the percentage of the reduction in deposited microorganism load at 6 ACH compared to 3 ACH ventilation rate was found to be 33% ± 25% near the inlet and 44% ± 32% near the outlet (Table 7:1).

7.3.3 The loss rate due to deposition onto surfaces

The loss rate of bioaerosols due to the deposition can be divided onto floor, walls and ceiling. For the limited time available and the restrictions in accessing the lab during the Covid-19 pandemic, only the loss rate due to deposition onto the floor surface was considered in the experiments in this chapter. To estimate the overall contribution by deposition, the deposition rate of bioaerosols on the ceiling and walls was substituted by a percentage equal to 23% and 44% of the deposited microorganisms' concentration on the floor, respectively based on Liu et al., (2020). Although this allows an estimation the inlet and outlet for ventilation were located in the ceiling for Liu et al., (2020) which may change the surface deposition pattern.

Assuming that the floor and the ceiling have the same surface area, equation 7.2 uses the same principle of equation 4.6 and calculates the loss rate due to deposition taking into account the deposition onto the walls and ceiling as a percentage of the deposition on the floor (real data from the experiment).

$$\lambda_d = \lambda_{d,f} + \beta_w \lambda_{d,f} \frac{2H\,(L+W)}{LW} + \beta_c \lambda_{d,f} \qquad (7.2),$$

Where, λ_d is the loss rate due to deposition onto all chamber surfaces (h^{-1}), $\lambda_{d,f}$, is the loss rate due to deposition onto the floor surface (h^{-1}), β_w and β_c are the percentages of C_{sf} , which is the indoor deposited microorganisms' concentration on the floor ($cfu.m^{-2}.h^{-1}$) for deposition on the walls and ceiling, respectively.

To calculate the loss rate of airborne microorganisms due to deposition onto the floor. Equation (4.7) can be used as demonstrated in chapter 4.

Equation (5.2) can be used to determine the deposition rate coefficient during passive sampling for each time frame (15 minutes). The deposition coefficient of *Staphylococcus aureus* for both locations (near the inlet and near the outlet) was found to be 0.60±0.33 (0.09 - 1.69) h^{-1}. These results fall within the same range of the previous work that suggested 0.10-0.80 (h−1) for different particle sizes of 0.55 to 1.91 µm diameter with varying settings of fan at speeds (0, 5.4, 14.2 and 19.1 $cm.s^{-1}$) in the laboratory room of 14.2 m^3 volume (Thatcher, et al., 2002; Lai, et al., 2012). However, it is higher than what was reported by Lai et al. (2012), which found that the deposition coefficient of *staphylococcus aureus* is 0.14 h^{-1} at a ventilation rate of 1.7 – 18 ACH conducted in a Class II biological safety cabinet. Both previous studies assumed that all the particles deposit only on the floor without considering deposition on walls and ceiling.

Using equation 7.2, the total loss rate due to deposition on all surfaces λ_d is 1.38 (h^{-1}). Figure 7:8 presents $\lambda_{d,f}$ as a box plot in different positions (Near the inlet or near the outlet) at different ventilation rates (3 or 6 ACH). There is no significant difference between the loss rate due to deposition on floor surfaces at different locations and ventilation rates which explains how the microorganisms in the air and on surfaces are related. Figure 7:8 represents the value of $\lambda_{d,f}$ which is the ratio between air and surface concentration, Figure 7:4 and Figure 7:7 show that the concentration of microorganisms is higher near the outlet and higher at the 3 ACH ventilation rate in the air and on surfaces, respectively.

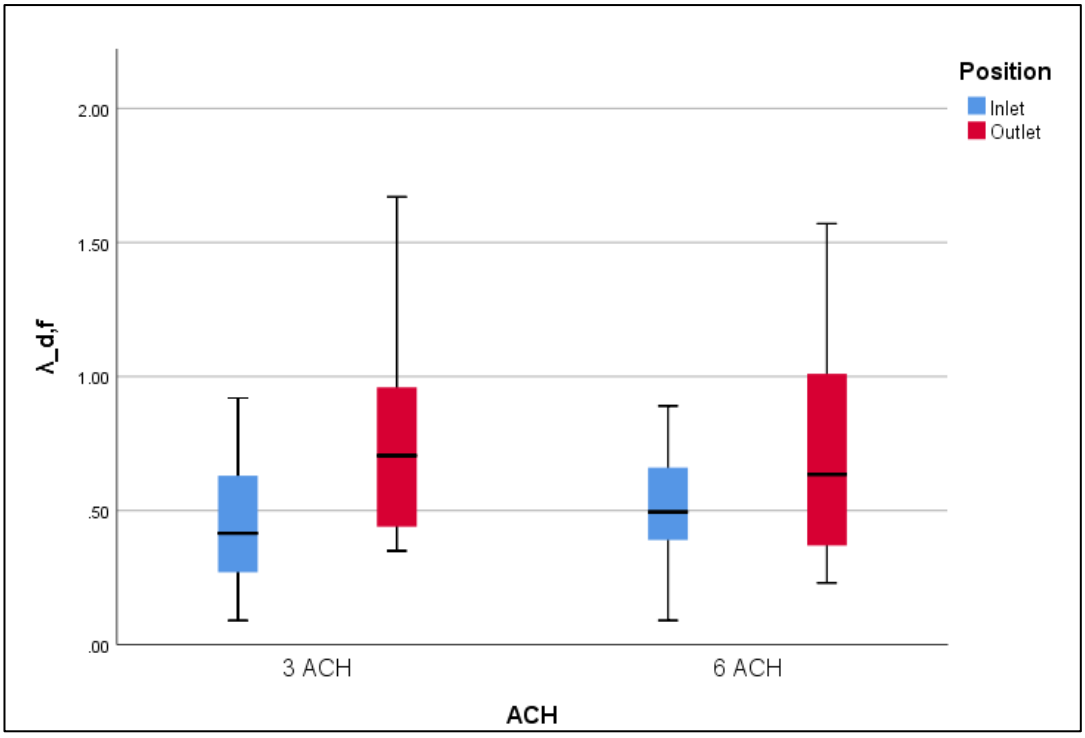

Figure 7:8 The boxplot of the total loss rate due to deposition on floor surfaces (h^{-1}) with different ventilation rate and positions.

Staphylococcus aureus (ATCC 6538) was used in this experiment is a spherical microorganism and has a diameter of 0.8-1.2µm as shown in Figure 7:9. This is particularly important to know in order to be able to compare with other experiments that report data for a range of particle sizes. As mentioned in chapter 4 (Table 4:2), the two researchers who previously did experiments for particles and *Staphylococcus aureus* in a controlled environment found that the loss rate due to deposition onto surfaces was within the range of results found in the experiments in this chapter or slightly higher. However, in the real world, the loss rate due to deposition onto the floor surface has been found to be 5 to 10 times higher; it was 2.77 in the microbiological office and 5.5 in the ICU reported by Wong et al.(2011). As shown in chapter 5, the loss rate measured in this study due to deposition onto the floor surface of a 4-bed ward was 3±2 for *Staphylococcus spp*. and 5±2 for ACC.

The real-word environments and especially hospital environments usually face a complexity of interactions between several environmental and behavioural factors (Hiwar et al., 2021) and the air and surface concentrations. A positive significant correlation was previously found

between the number of particles with a diameter of >10 μm and the bioaerosols loading, which leads to an effect on the rate microorganisms are deposited on open petri dishes (Mousavi et al., 2019). Although, the diameter of *Staphylococcus aureus* is known to be about 1μm, this doesn't mean that the aerosolised droplet in the chamber, that carries the *Staphylococcus aureus* has the same size; the distribution of the nebuliser particle size range is 0.3-5 micron. In a hospital environment microorganisms, including *Staphylococcus spp*, may be carried on larger particles such as skin squame (Pankhurst et al., 2011).

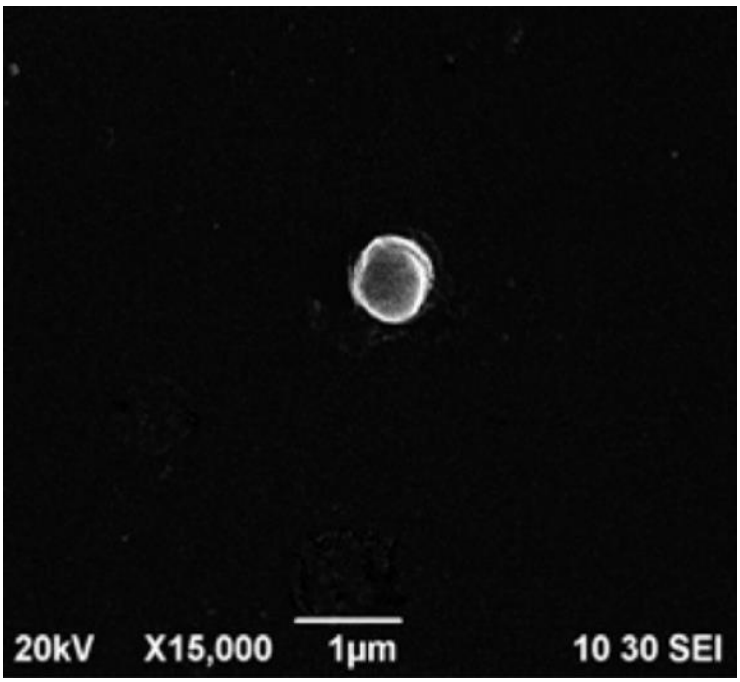

Figure 7:9 The *Staphylococcus aureus* diameter (Lai et al., 2012)

7.3.4 The generation rate efficiency (nebuliser)

According to Table 7:1, the mean concentration of bioaerosols load during the experiments at 3 ACH sampled at the collection point near the inlet air and the collection point near the outlet air were 3797 cfu/m^3 and 5599 cfu/m^3 respectively. The mean concentration of bioaerosols load at 6 ACH near collection point at inlet air and near collection point at outlet air were 2218 cfu/m^3 and 3167 cfu/m^3 respectively.

Equation 6.2 is rewritten as equation 7.3 to find the value of β considering λ_d as 1.38 (h^{-1}).

$$\beta = \frac{\left(C - C_0\, e^{-(\lambda_v+\lambda_d)t} \right)\ (\lambda_v + \lambda_d)\, V}{q\ (\ 1 -\ e^{-(\lambda_v+\lambda_d)t})} \qquad (7.3)$$

Figure 7:10 shows the bioaerosols load in the real experiments and the load calculated mathematically under the steady-state conditions considering three different scenarios. The first scenario considers the ventilation rate at 3 ACH as the only cause for the loss rate. The second scenario considers the ventilation rate in addition to the loss rate due to deposition, which decreases the gap from the expected value, which corresponds to the real experiments data. The third scenario considers the ventilation rate 3 (h^{-1}), the loss rate due to deposition (1.38 h^{-1}), and the efficiency of the nebuliser (50% ± 13%). Figure 7:11 show the same three scenarios under with 6 ACH.

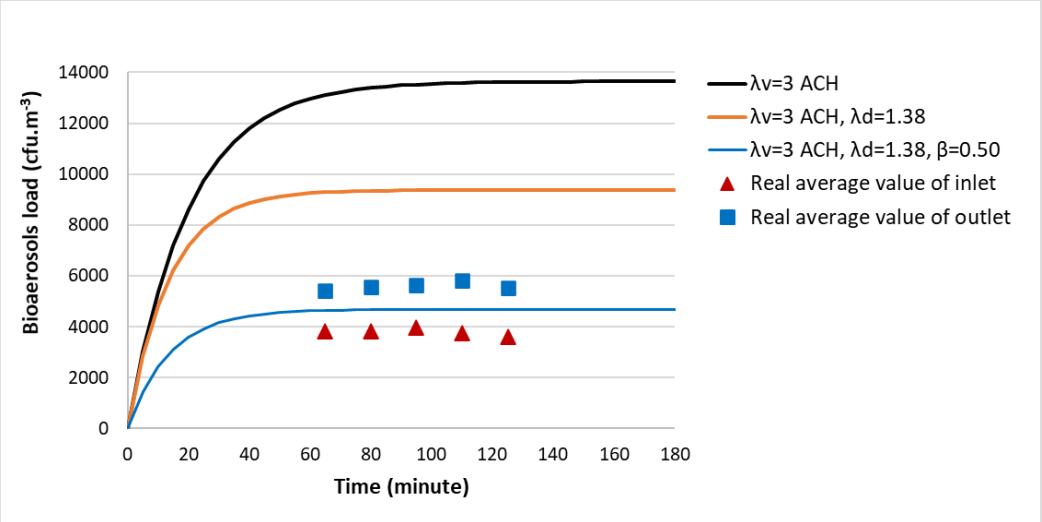

Figure 7:10: The bioaerosol load under the steady-state conditions considering a ventilation rate of 3 ACH, deposition loss rate and the nebuliser's efficiency.

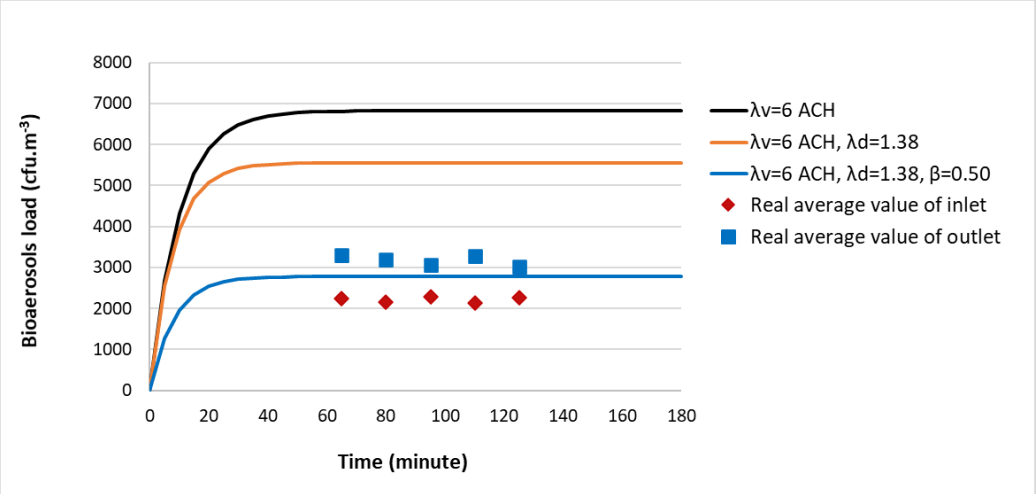

Figure 7:11 The bioaerosol load under the steady-state conditions considering a ventilation rate of 6 ACH, deposition loss rate and the nebuliser's efficiency.

7.4 Comparisons and Limitations

7.4.1 Limitations

The results shown in this chapter highlight a number of benefits and limitations. This work contributes to the research community as very few experiments have performed sampling over time and measured either particles or the concentration of microorganisms in the air and on surfaces directly together. Previous studies with microorganisms are generally in smaller chambers (Lai et al., 2012) or in real-world settings (Wong et al., 2011; Smith et al., 2018a). The controlled chamber enables us carry out room-scale studies while also controlling several variables. However, some variables are still unknown including the efficiency of the nebulizer and the sampler which need to be calculated based on the results obtained or estimated by further assumptions. These experiments are complex and have several variables that need to be considered. A novel device was developed to enable these experiments and to perform time series surface sampling alongside to the air sampling. The results show that there is a difference in concentration at different spatial locations. However, the experiments considered only two locations which were expected to reflect the lowest and highest concentrations in the room. Previous work shows the variation at

more locations through measurements and CFD, but don't directly sample air and surface together (King et al., 2017b).

It was not possible to investigate the effect of particle sizes on the deposition rate of bioaerosols on surfaces due to the limited time and the COVID-19 restrictions imposed on the access to the lab and university resources. It can be important to investigate this in future work to better understand the factors that affect deposition, and particularly to evaluate the deposition when microorganisms are carried in larger aerosols or when using a different type of microorganism especially if it had a different size like Bacteriophage MS2 (~0.028 micron) or different shape like *Escherichia coli* (rod-shaped rather than spherical). Another useful investigation is to assess the effect of changing the furniture (surfaces), the ventilation regime (The position of ventilation grilles), the temperature and humidity, and the presence of heat sources in the room. The meta-analysis study in chapter 3 suggests there may be relationships between environmental factors and the behaviour of microorganisms, with particle mass concentration and ventilation rate (CO_2 level) both positively correlated.

7.4.2 Comparison between environments

One of the principal benefits of studying the relationship between microorganisms in the air and on surfaces and understanding the factors that affect this relationship in a hospital environment, is to enable healthcare workers to control these factors and improve the environmental conditions to minimise infection risk. This relationship reflects the loss rate due to deposition on surfaces, which can be used in an infection risk model such as that for COVID-19 by Miller et al. (2021).

In the hospital environment, the loss rate due to deposition onto surfaces based on the multilinear regression model (Chapter 4) from the data in (Wong et al., 2011) was 3.98 h^{-1} in the microbiology office and 8.02 h^{-1} in the ICU; our hospital study (Chapter 5) found that it was 4.32 ± 2.88 h^{-1} for *Staphylococcus spp.* and was 7.2 ± 2.88 h^{-1} for ACC. However, in the controlled environment (Chapter 7) it was 1.38 ± 0.48 h^{-1} for *Staphylococcus aureus* (Table 7.6). The results show that the loss rate due to deposition $\lambda_{d,f}$ in the hospital environment is higher than that in the controlled

environment. The previous studies, which was performed in a controlled environment, found that λ_d is less than 1 h^{-1} (Thatcher, et al., 2002; Lai et al., 2012).This could be explained by the effect induced by the difference in the particle number and size in the air, the difference in people presence level and difference in the type of microorganisms.

The HCWs presence level was considered in the multilinear regression model presented in chapter 4, and it was found that using data from (Wong et al., 2011) in the microbiological office with the windows closed, no ventilation in place and with people level of 2 the deposition rate was 3.98 h^{-1} while it was 8.02 h^{-1} in the ICU with mechanical ventilation of 12 ACH and regular number of HCWs present. The transient relationships between air and surface bioburden for different type of microorganisms (in chapter 5) showed that *Staphylococcus spp* has a smaller λ_d than that of ACC in the practical hospital study. A change in the ventilation rate affects the concentration of microorganisms in the air and on surfaces as demonstrated in chapter 7. However, the loss rate due to deposition is not affected by the ventilation rate as it is a ratio between the concentration of microorganisms in the air and on surfaces as shown in equation 4.7 and Figure 7:8.

The loss rate due to deposition onto surfaces should be considered in airborne infection risk models, as it is a significant factor even for small aerosols such as in the chamber experiments carried out here. It is likely that it can be assumed as a constant value even if the ventilation rate changes. However, it may change when the occupancy of the room or the number and size of particles changes. The knowledge that environmental factors have a significant impact on the concentration of microorganisms in the air and on surfaces, and the fact that these factors can be controlled to mitigate the infection risk means that measures can be taken to prevent the level of microorganisms to breach the accepted threshold by these factors and employing efficient cleaning and ventilation systems. Also, a well-designed ventilation system must be installed to maintain a healthy environment and to enable quick recovery in case of a breach of the accepted level of microorganisms.

Table 7:6 A summary of studies concerning the loss rate due to deposition onto surfaces

Based on	People present level	Ventilation type and rate ACH	Type of microorganisms	SAR	$\lambda_{d.f}$ h^{-1}	λ_{d} h^{-1}
Wong et al., 2011 (Hospital study)	2	Natural (Closed window)	ACC	8.31	2.77	3.98
	N/A	Mechanical (12 ACH)	ACC	16.71	5.57	8.02
Hospital study (Chapter 5)	88 ± 21	Natural (window was open)	ACC	14 ± 6	5 ± 2	7.2 ± 2.88
	88 ± 21	Natural (window was open)	*Staphylococcus spp*	9 ± 5	3 ± 2	4.32 ± 2.88
Thatcher, et al., 2002 (controlled environment)	0	Laboratory room (14.2 m^3) varying settings fan at speeds (0, 5.4, 14.2 and 19.1 cm.s-1)	*Staphylococcus aureus*	1.2 ± 0.72	0.5 ± 0.3	N/A
Lai et al., 2012 (controlled environment)	0	Class II biological cabinet (0.07 m^3)/ Mechanical (1.7 – 18 ACH)	*Staphylococcus aureus*	0.04	0.14	N/A
Chamber study (Chapter 7)	0	Chamber room (32 m^3)/ Mechanical (3 or 6 ACH)	*Staphylococcus aureus*	1.36 ± 0.75	0.6 ± 0.33	1.38 ± 0.48

The number of people performing an activity within 15 minutes, multiplied by the actual duration time they were in the.

SAR is the Surface air ratio Surface (m. h^{-1}), which calculated based on equation 4.5

$\lambda_{d.f}$ (h^{-1}) calculated based on equation 4.7

λ_{d} (h^{-1}) calculated based on equation 7.2

7.5 Summary

This chapter investigates the effect of ventilation rate on the deposition rate of microorganisms under the steady-state conditions in a controlled microbiological chamber. It presents mathematical equations to estimate the concentration of bioaerosols in varying scenarios and to examine the effect of the ventilation, deposition and the efficiency of the nebuliser on the measured microbial load in air and on surfaces.

For the spatial deposition rate within the chamber, all the experiments show that the collection point near the outlet has a significantly higher bioaerosol load and a significantly higher number of deposited microorganisms compared to the collection point near the inlet. This likely reflects the effect of the air flow pattern in the room and highlights that even in a room with good ventilation there can be significant spatial differences in concentration in air and on surfaces.

The results show that increasing the ventilation rate from 3 ACH to 6 ACH results in a reduction of bioaerosols load in air by 43% ± 8% and 45% ± 10% when sampling near the inlet and the outlet, respectively. Furthermore, this increase in the ventilation rate reduces the deposited microorganism load by 33% ± 25% and 44% ± 32% when sampled near the inlet and the outlet, respectively.

This chapter also investigates the loss rate due to deposition on the floor ($\lambda_{d,f}$) for both locations near the inlet and near the outlet which was found to be 0.60 ± 0.33 (0.09 - 1.69) h^{-1}, this result agrees to the literature and confirms the influence of deposition loss rate on the bioaerosol load. However, to obtain more realistic data, the deposition on the walls and ceiling was also considered as a percentage of floor deposition to find the total deposition on all surfaces (λ_d) that is more realistic but slightly higher (1.38 h^{-1}) than what the literature suggests. For comparison with the real-world data, the loss rate due to deposition on the floor was lower than what was found in the literature and lower than our findings in the hospital study in chapter 5.

The efficiency of the microorganism generation from the nebuliser was calculated based on mathematical equations using data from the practical experiments and was found to be 50 % ± 13% for 3 ACH and 6 ACH ventilation rates.

Finally, this chapter shows that when considering the ventilation rate, the loss rate due to deposition, and the efficiency of the nebuliser, the mathematical calculations conform to the practical experiment results obtained using the AMPAS device.

8 Conclusions, future work, and implications of the research

Hospital acquired infections (HAIs) are infections that occur during the stay inside a hospital or another healthcare facility. These infections lead to increased death rates and higher costs for treatment from hospital admission to follow-ups and medication. The infection that accounts for the highest percentage (22.8%) of all HAIs is the respiratory tract infection usually caused by several pathogens, commonly including *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis,* and *Staphylococcus aureus*. The total aerobic colony count (ACC) is widely used as a proxy for infection risk, and a benchmark of 2.5 cfu.cm^2 has been suggested for environment cleaning. Alongside HAIs, the COVID-19 pandemic has raised awareness of the need to understand respiratory disease transmission and the relative importance of microorganisms in the air and on surfaces. The literature presents several studies showing environmental and physical factors that influence the microbial load in the air and on the surfaces from real-world and controlled environments, but despite this there is a lack of quantitative data or consolidation of the prior work in this area.

This research aimed to investigate in detail the factors that influence the relationships between microorganism loads in the air and on the surfaces. This was achieved by conducting a systematic literature review and meta-analysis to bring together information from prior work in a quantitative way, followed by experimental measurements of microorganisms in air and on surfaces that was collected using a novel surface sampling device in a variety of environments coupled with mathematical analysis.

The key findings of this research are summarised below. The implications of the findings are discussed, and potential areas for future research are highlighted.

8.1 Conclusions

The main observations and conclusions of this work are summarised and presented here to provide an insight into the results obtained throughout this research.

8.1.1 The prevalence of airborne microorganisms in hospital environments

The presence of microorganisms in the air and their potential to cause infection has long been a concern for healthcare workers and researchers. The concentration of airborne microorganisms in healthcare environments and the parameters that influence it have been studied for decades. It is particularly important to characterise the factors that influence the airborne microorganisms load and their interaction with each other in order to be able to improve the wellbeing of patients, visitors and health workers by controlling the environmental parameters. Several of these studies have reported that there is a positive correlation between the concentration of indoor airborne microorganisms and infection risk in different hospital wards. This is because airborne microorganisms can either directly transfer to the patient, or deposit on surfaces and then transfer (indirectly) to the patient through hand touch. Another parameter is the status of patients which was highlighted by several studies showing that being colonised, not colonised or infected has a significant impact on the bioaerosol load for different types of microorganisms including bacteria (*S. aureus*, ACC), fungi (Aspergillus fumigatus, Aspergillus spp and total airborne fungi) , and viruses. However, findings across these multiple studies have not been compared or consolidated in a consistent way with the indoor hospital environment parameters. A systematic literature review was conducted to highlight the gaps in research and the following results were observed:

- There are only a small number of studies that report the concentration of airborne microorganisms in hospital environments with quantitative data while also measuring the environment and activity factors.

- There is not enough data in the literature to identify and quantify the factors that have a high impact on the concentration of microorganisms in the air.
- There are conflicting results in the literature on the relationships between IAQ parameters (temperature, relative humidity, CO_2, particle mass concentration and particulate matter of size) and microorganism concentrations in the air.
- There is a lack of guidelines for air sampling in healthcare settings leading to inconsistent results. We provided recommendations for researchers to collect quantitative results in a unified approach considering human activities and environmental factors.
- Quantitative studies enable researchers to perform a meta-analysis study and to accurately investigate the relationship between IAQ parameters in the hospital and their impact on the concentration of airborne microorganisms.

8.1.2 The relationship between indoor air quality parameters and airborne microorganisms in hospital environments.

Controlling IAQ parameters can potentially influence the concentration of airborne microorganisms which could lead to an increase or a reduction in the infection risk. The correlation between IAQ parameters and the concentration of airborne microorganisms in a hospital environment was studied using a meta-analysis approach. A directed acyclic graph (DAG) methodology was used to explain the interaction of different IAQ parameters. Understanding these parameters and their interaction with each other is particularly important as it informs healthcare workers how to utilise them to reduce infection risk. The meta-analysis was performed on the data collated from the literature, including all studies that provide quantitative data on airborne microorganisms collected in hospital environments, excluding areas with specialised ventilation like operating rooms. The results found using the meta-analysis led to the following conclusions:

1- There is a significant moderately positive correlation between airborne bacterial load ACC and airborne fungal load TF r=0.31 (95% CI=

[0.07; 0.52]), P=0.014. This will help to understand that the releasing of bioaerosols may come from the same source and that ACC can be generalised to reflect other types of microorganisms.

2- There is a significant positive correlation between ACC and temperature, CO_2, particulate matter size and concentration ≤5μg/m^3. Controlling the IAQ parameters and using these parameters in real time could help to reduce the concentration of airborne microorganisms which leads to mitigating the infection risk.
3- There is insufficient data to demonstrate clear correlations between ACC and relative humidity and between TF and IAQ parameters. Taking each parameter separately could lead to misleading results, so all parameters must be considered to get the best results.
4- There is a need for more studies on the relationship between IAQ parameters and the concentration of microorganisms in hospital environments. These studies can be used for modelling infection risk.

8.1.3 The relationship between airborne microorganisms and surface bioburden in hospital environments

The environment is known to contribute to the transmission of HAISs and surface contamination is likely to be one of the risk factors. The literature shows that several air and surface sampling techniques are used in hospital environments. It also shows that passive sampling can be used to estimate the concentration of airborne microorganisms. Existing mathematical equations that describe the relationship between airborne microorganisms and surface bioburden were evaluated, and the following observations were made:

1- The predictions of both existing equations were significantly correlated with the actual data collated from the literature.
2- A multilinear regression model based on data from literature was developed to consider the activity level when predicting the airborne microbial load. This multilinear regression model provides predictions that are significantly better than the simple linear model and the two existing equations (both based on literature data).

3- The literature does not provide recommendations on the accepted level of airborne microorganisms. Our multi-regression model recommends that the max accepted level of airborne microorganisms in a hospital ward is 118 cfu.m^{-3}. This value is based on the results found on surfaces meaning that 2.5 cfu/cm^3 should not be exceeded. The benchmark value of microorganisms on a surface is suggested to be less than 5 cfu.cm^{-2} to minimise the infection risk. Cleaning in hospital wards usually occurs at least every 24 hours; thus, the deposited microorganisms on surfaces within 1 hour (y) should not exceed 0.208 cfu.cm^{-2}.h^{-1} (5 cfu.cm^{-2} /24 h). Then, our multiple linear regression model in equation (4.10) can be used to find the average concentration of airborne microorganisms in the hospital wards equation.
4- More data is needed on measuring the concentration of airborne microorganisms and surface bioburden using active and passive samplers, considering the activity level of healthcare workers.
5- Collected data should provide information on a sufficient period rather than only representing a snapshot to reflect the impact of the healthcare workers' activities and other contributing factors more reliably.

8.1.4 Measuring airborne microorganisms and surface bioburden in a hospital environment

Analysis of published data identified that there is a lack of time-series data on the transient relationship between airborne and surface microbial load, with considerations to the presence and activity level of healthcare workers. We measured the deposition rate of airborne microorganisms on surfaces over time in a hospital respiratory ward and considered the cleanliness threshold to evaluate the fluctuations in microbial load and to provide recommendations on the accepted level of airborne microorganisms. A mathematical equation is introduced to describe the concentration of airborne and surface microbial load in terms of cfu/m^3, to enable the comparison between results obtained using both active and passive

sampling techniques. The deposition rate on surfaces in different wards was studied, and the following observations were made:

1- The average concentration of airborne microorganisms measured for ACC was 196±103 cfu/m^3, which is significantly correlated with *Staphylococcus spp,* that represented (86%±35%) of ACC.
2- The IAQ parameters were all within the range of recommended values for a hospital settting, including temperature (19±2°C), relative humidity (50% ± 7%), Carbon dioxide level (644 ±115 ppm), particle mass concentration 2.5 µg/m^3 (19±32 µg/m^3), and particle mass concentration 10 µg/m^3 (26±44 µg/m^3).
3- The airborne ACC load was significantly correlated with relative humidity, CO_2 and particle mass concentration of 10 µg/m^3, while it was not significantly correlated with temperature or particle mass concentration of 2.5 µg/m^3. These results correspond to the findings of the meta-analysis study.
4- The ACC load in the air had a weak negative significant correlation with the number of patients, and it was not significantly correlated with the number of visitors and HCWs and their activities. The *Staphylococcus spp*. load in the air was significantly correlated with the number of HCWs and the bed bathing activities, but it was not significantly correlated with the number of patients and visitors.
5- In the single-bed room, the average concentration of ACC and *Staphylococcus spp.* Was lower than that of the four-bed and ten-bed wards. Also, the concentration of ACC and *Staphylococcus spp.* was higher in the morning (before cleaning) than in the evening (four hours after cleaning).
6- All the samples in the single-bed room were within a recommended benchmark level for surface contamination of 2.5 cfu.cm^{-2}, while the four-bed and the ten-bed wards had 16% and 27% of samples over the threshold four hours after cleaning, respectively.
7- The contamination at the patient notes, tray, and table was significantly higher than the contamination at the right bed-rail and the left bed-rail. The contribution of the airborne microorganisms' load to

the total surface microbial load was calculated to be 65%± 18 four hours after cleaning.

8- We provided a mathematical equation (simple model) to link the airborne concentration to the surface load and to express results obtained using passive samplers in volumetric terms (cfu.m^{-3}). This is based on the simple linear model of data from the four-bed respiratory ward experiment, which needs external validation to consider the presence and activity level of HCWs, while the equation in chapter 4 considered the HCWs level using the multi-regression model.

9- The loss rate due to deposition onto floor surfaces was 3±2 h^{-1} for *Staphylococcus spp*. and 5±2 h^{-1} for ACC.

10- With the large number of factors and the fluctuation in some of the results, more data needs to be collected in a controlled environment to obtain more reliable results and to understand the influence of ventilation on the deposition rate on surfaces.

8.1.5 Introducing an automated multiplate passive air sampler (AMPAS) to measure the deposition rate of airborne microorganisms over time

A novel configurable device that can expose a plate to air for a pre-determined interval, cover it, and autonomously expose a different one was designed, implemented, and validated to gain time series surface sampling without human intervention. The validation of the AMPAS device included ensuring that it could perform sampling without undesired contamination and spill over. It also included checking whether the results obtained using AMPAS are consistent across the six plates of Ø 55 mm and with those obtained separately using open Petri dishes of Ø 90 mm. After using and validating the AMPAS device in different environments, the following points were concluded:

1. Results show that AMPAS is a valuable tool that can be used in controlled and non-controlled environments to provide consistent passive sampling results. It enables the sampling of airborne microorganisms over time using pre-configured settings without the

need for human intervention, which protects both data and humans from undesired contamination.

2. AMPAS can provide automated time-series surface samples and making it possible to investigate the influence of ventilation rate in spatiotemporal bioaerosols under the steady-state condition.
3. The AMPAS can collect samples in areas with low and high concentrations of microorganisms and can thus be used in controlled environments and real-world environments with reliable results.

8.1.6 Deposition rate of microorganisms under the steady-state condition in a controlled microbiological chamber

The loss rate due to deposition onto surfaces is quantified considering the ventilation rate and different locations in the room. The AMPAS device was used together with an Anderson impactor to collect samples from surfaces and air respectively, and mathematical equations were used to estimate the microorganism concentration and the deposition rate in different scenarios under steady-state conditions. The data collected led to the following conclusions:

1- Within the controlled bioaerosol chamber, there is a significantly higher bioaerosols load and higher deposited microorganisms at the collection point near the extract air (outlet) than the collection point near the supply air (inlet).

2- Increasing the ventilation rate from 3 to 6 ACH reduces the bioaerosols load and the deposited microorganism load by 44% ± 9% and 29% ± 28.5%, respectively.

3- The results of loss rate due to deposition on the floor (0.60±0.33 h^{-1}) agree with experiments in controlled environments reported in the literature. However, considering the deposition on the walls and ceiling leads to more realistic data, where the loss rate due to deposition was estimated as 1.38 h^{-1} which is slightly higher than suggested in the literature. These results were still lower than deposition rates found in hospital sampling, which was 3±2 h^{-1} for *Staphylococcus spp.* and 5±2 h^{-1} for ACC.

4- The efficiency of the microorganism's generation from the nebuiliser was estimated to be around 49%.
5- Considering the ventilation rate, the loss rate due to deposition, and the efficiency of the nebuliser, the mathematical calculations agree with the results of the practical experiments obtained using the AMPAS device.

The general conclusions drawn from across all the elements of the study indicate that the relationship between microorganisms in the air and on surfaces is a significant parameter that needs to be considered in airborne and surface infection risk models. This relationship can be represented by the loss rate due to deposition onto surfaces which is affected by the occupancy of the room or the number, size of particles changes and the difference in the type of microorganisms. Increasing the ventilation rate from 3ACH to 6ACH in the chamber reduces the concentration of microorganisms in the air and on surfaces by 40%. Since the decrease in concentration occurs in both airborne and deposited microorganisms, the loss rate due to deposition was shown to remain constant as the ventilation rate increases. This means that controlling the ventilation rate and the environmental parameters can mitigate the infection risk through both air and surface contamination in the hospital environment. Defining a single value for deposition rate is not feasible, but this study together with previous data, provides a realistic range of values for models and more insight into the factors that affect the rate. Also, the study has developed and demonstrated methods for assessing air and surface contamination together that can be effectively deployed in future studies.

8.2 Implications

By understanding the influence of IAQ parameters in the hospital and their interaction with each other, controlling them becomes an efficient measure to reduce the infection risk. The meta-analysis study highlights the impact of having an accurate predictive model and suggests it can be invaluable in monitoring airborne microorganisms' concentration in real time. To provide an easy and cost-effective method to use in a healthcare setting,

mathematical equations were proposed to enable researchers to express surface microorganisms load that are deposited on open petri dishes in terms of cfu/m^3, which makes it possible to widely use settle plates. The recommended accepted level of airborne microorganisms in the hospital is proposed by using the multi-regression model. This enables healthcare workers to plan their ventilation and cleaning strategy in an improved manner.

The AMPAS device is likely to provide a benefit for researchers and those who are sampling in hospital settings as part of infection control strategies. For the chamber study, the design of the AMPAS device is provided with all of its components so that researchers can use it to efficiently obtain time series surface sampling data without the need for human intervention.

Designing an effective model can be achieved by collecting more information on the concentration of microorganisms and by feeding the model with real-time data on the indoor air quality parameters. Even more realistic results can be obtained by using the mathematical equations provided to consider the ventilation rate, the loss rate due to deposition on all surfaces, including walls and ceiling, and efficiency of the nebuliser, without the need to make false assumptions.

8.3 Future work

The results and the conclusions produced in this work have contributed to the current knowledge and have also generated mathematical equations in addition to valuable quantitative data on the relationship between airborne microorganisms, surface deposited load, and the factors that influence this relationship. We have also identified gaps in knowledge where more studies can be utilised to enrich the research and to tackle the issues that are still unknown. Some of the areas that need to be investigated are:

8.3.1 Time series data

More experiments are needed with detailed sampling results considering IAQ parameters while observing the activities of healthcare workers to gain a better understanding of the time-series fluctuation data on the transient

relationship between airborne and surface microbial load at hospitals in different locations and during varying seasons. These experiments can provide a better understanding of the impact of the time of the year and the geographical location on the concentration of microorganisms in the hospital environment. This can be achieved by collecting more environmental samples with the same approach used in chapter 5. These samples should be collected at different places and during other times of the year to understand if the relationships that we have found would hold more widely. This validation was not made during this study due to the restrictions imposed by the COVID-19 pandemic. Another valuable research is to use microorganisms other than ACC, such as fungi and viruses and compare the findings with ACC.

8.3.2 Influence of particle size and environmental parameters

More studies are needed to investigate the effect of different dry particle sizes, locations, the effect of ventilation system grille positions, the effect of temperature and humidity and the effect of furniture on the deposition rate of airborne microorganisms on surfaces in a controlled environment. Beato Arribas et al., (2015) only looked at the air, but showed a difference in airborne concentrations with ventilation position relative to the furniture in the room. Previous studies have shown that layout of the room, ventilation pattern and heat sources can be significant to deposition rate and have shown that the survival of microorganisms are affected by factors including temperature and humidity (King et al., 2013; Wilson et al., 2021).

These investigations can help to understand the inconsistency of the deposition rate of microorganisms results in the controlled chamber and in the real-world hospital environment. Some of these experiments were part of the plan for this research but did not happen due to the restrictions imposed by the COVID-19 pandemic.

8.3.3 Influence of microorganism

Our study has focused on ACC and *Staphylococcus aureus*. However, it would be worth looking at other bacteria that cause a problem in healthcare such as *Pseudomonas aeruginosa* (known to be aerosolised from sinks and drains), *Clostridium difficile* (as been shown to be dispersed in the air) as

well as viruses (Booth et al., 2005; Panagea et al., 2005; Faires et al., 2012). Although there is growing data on COVID, there is still very little information on any other viruses. There is a growing data on COVID but since viruses are much smaller in diameter and so may deposit more slowly, they are difficult to study in both the real world and in a chamber setting, so proxy experiments may need to use a phage (phi6) (Vatter et al., 2021).

8.3.4 Mitigation strategies

Using air cleaning devices including portable HEPA filters, Ultraviolet C (UV-C) devices, and more recently the potential for far-UV devices has been widely suggested as a means of controlling airborne transmission of infection. Studies to investigate the influence of these methods on the bioaerosols load and the deposited microorganisms load to mitigate the infection risk would be beneficial. Although studies consider airborne microorganisms' concentration, there are still very few studies that consider air and surface together. For this reason, we have recently carried out a study on using far-UVC to mitigate the infection risk (Eadie et al., 2021).

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