What is the relationship between indoor air quality parameters and airborne microorganisms in hospital environments? A systematic review and meta-analysis

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Abstract

Airborne microorganisms in hospitals have been associated with several hospital-acquired infections (HAIs), and various measures of indoor air quality (IAQ) parameters such as temperature, relative humidity, carbon dioxide (CO₂), particle mass concentration, and particle size have been linked to pathogen survival or mitigation of pathogen spread. To investigate whether there are quantitative relationships between the concentration of airborne microorganisms and the IAQ in the hospital environment. Web of Science, Scopus and PubMed databases were searched for studies reporting airborne microbial levels and any IAQ parameter(s) in hospital environments, from database inception to October 2020. Pooled effect estimates were determined via random-effects models. Seventeen of 654 studies were eligible for the meta-analysis. The concentration of airborne microbial measured as aerobic colony count (ACC) was significantly correlated with temperature (r = 0.25 [95% CI = 0.06–0.42], p = 0.01), CO₂ concentration (r = 0.53 [95% CI = 0.40–0.64], p < 0.001), particle mass concentration (≤5 µg/m³; r = 0.40 [95% CI = 0.04–0.66], p = 0.03), and particle 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, while not being significantly correlated with relative humidity or particulate matter of size >5 µm. Conversely, airborne total fungi (TF) were not significantly correlated with temperature, relative humidity, or CO₂ level. However, there was a significant weak correlation between ACC and TF (r = 0.31 [95% CI = 0.07–0.52], p = 0.013). Although significant correlations exist between ACC and IAQ parameters, the relationship is not definitive; the IAQ parameters may affect the microorganisms but are not responsible for the presence of airborne microorganisms. Environmental parameters could be related to the generating source, survival, dispersion, and deposition rate of microorganisms. Future studies should record IAQ parameters and factors such as healthcare worker presence and the activities carried out such as cleaning, sanitizing, and disinfection protocols. Foot traffic would influence both the generation of microorganisms and their deposition rate onto surfaces in the hospital environment.
Hospital-acquired infections (HAIs) are a globally significant problem and their treatment can be costly. In the UK, HAIs are estimated to cost up to a billion pounds per year as of 2017 and the hospital environment is thought to play a role in approximately 20% of all HAIs by influencing the survival and spread of pathogens in the environment. The hospital environment is subject to workplace design and layout, operation and maintenance, and hosts multiple interactions between 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 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. A very small number of studies correlated virus concentrations to these factors, hence the focus of this study on the investigation of relationships between bacteria and fungi in the air and IAQ parameters. Surfaces, air, and indoor structure including ventilation systems have all been shown to act as reservoirs for pathogens, and in some cases, these pathogens can persist for months in a hospital environment. Previous studies have used the information from environmental sampling to link bioburden levels in the air, on surfaces bioburden and HAI rate.

Microbial sampling of the air can be used to evaluate the likely concentration of airborne microorganisms present in the hospital environment. The majority of studies apply culture-based methods to assess viable microorganisms. Airborne microbial load can be quantified by using either active or passive sampling methods. IAQ parameters such as temperature, relative humidity, CO₂ level (which reflects the ventilation rate), particle mass concentration, and particle size are important for the health and well-being 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 a particular concern. Many bacteria and fungi favor more humid conditions. However, there is evidence that virus survival increases at humidity below 40% RH. Guidance varies around the world, but temperatures within 16–25°C and humidity in the range 40%–60% RH are commonly recommended. CO₂ is related to the exhaled breath of occupants and is frequently measured in indoor environments as an indicator of ventilation rates. A number of studies have also shown that ventilation rates expressed through CO₂ concentrations can be used to evaluate airborne infection risk. Airborne particles provide a general measure of indoor air quality (IAQ) and can be related to indoor sources and activity or outdoor conditions. Some studies suggest using airborne particles as a proxy for cleanliness of the air, including to commissioning of specialized hospital ventilation systems. The directed acyclic graph (DAG) approach is a good way to investigate causality with variables with respect to confounding.

Although the correlation between IAQ parameters and microorganism prevalence and survival has been studied for decades, there are conflicting results 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 aim of this study is to carry out a systematic review and meta-analysis to investigate the relationships between the level of airborne microorganisms and IAQ parameters in a hospital environment. By bringing together data from multiple studies, the paper 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 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.
2 | METHODOLOGY

2.1 | Search and inclusion criteria

A systematic review was performed to identify relevant studies. For the identification phase, three electronic databases (Web of Science, 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 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. We excluded papers conducting air sampling in other types of healthcare buildings (e.g., G.P. surgery, clinic), in hospital rooms with specialist ventilation ≥10 air changes per hour (e.g., isolation rooms, operating theaters) or in areas undergoing construction or renovation. Studies with relevant data were included for the meta-analysis (Figure 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, CO₂, particle mass concentration (≤5 or >5 µg/m³), or particulate matter of size (≤5 or >5 µm) measured at the same time point.

The DAGitty and statistical software R 4.0.0 (package “ggdag” version 0.2.3) were used to build a DAG (Figure 2) to describe how potential confounders and the air quality parameters relate to microbial measures.

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 were 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 (1) was used to compute the correlation coefficient from multiple regression and the general linear model for taking covariates into account.\(^3\)

\[ r = \frac{t}{\sqrt{t^2 + df}} \quad \text{(1)} \]

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.\(^3\) Sensitivity analysis of the meta-analyses to detect potentially outlying studies was performed using visual approaches including (1) externally standardized 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.\(^3\) 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: \(\tau^2 = 0\). 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.\(^3\) 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 generalize beyond the studies included in the meta-analysis.\(^3\) Forest plots were used to visualize the overall estimates of the study effects with corresponding confidence intervals.\(^3\) This systematic review and meta-analysis was performed according to the Preferred Reporting Items for the Systematic Reviews and Meta-Analysis (PRISMA) guidance.\(^3\) 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 (Appendix S1).

3 | RESULTS

A total of 1173 studies were retrieved and 654 studies screened after duplicates were removed. After screening through titles and abstracts, 197 studies remained for full text assessed for eligibility. Seventeen studies were included in the final meta-analysis (Figure 1). These presented quantitative airborne microbial concentration measured as ACC or airborne TF 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 1).\(^1\) 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. A DAG approach was used to identify possible confounders within the data structure and which variables need to be included (Figure 2).\(^3\) 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.

3.1 | Correlation between airborne microorganisms and ambient air temperature

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/m³ and TF 4–1125 cfu/m³. As shown in Figure 3A, 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 to 80 with a total of 300 values over all studies.\(^{2,7,10,11,28,30}\) TF was not significantly correlated with temperature \(r = 0.05\) [95% CI = −0.12–0.21], \(p = 0.60\) with a sample size ranging from 12 to 80 and a total of 154 values (Figure 3B).\(^{2,10,11,28,30,46}\)

3.2 | Correlation between airborne microorganisms and ambient relative humidity

Eight studies provided data for measured relative humidity alongside microbial concentrations in air. Relative humidity reported in the studies ranged from 17% to 79% with microbial concentrations ACC in the range 20–6295 cfu/m³ and TF 4–1125 cfu/m³. Relative humidity was not significantly correlated with ACC \(r = 0.06\) [95% CI = −0.15–0.27], \(p = 0.59\) with a sample size ranging from 12 to 80 and a total of 333 values (Figure 4A).\(^{2,7,10,11,28,30,32}\) There was also no correlation with TF \(r = 0.07\) [95% CI = −0.16–0.28], \(p = 0.56\) with a sample size ranging from 12 to 103 and a total of 368 values (Figure 4B).\(^{2,7,10,12,28,30,32,46}\)

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 CO\(_2\) concentration range was 470–1022 ppm above background, TF level 11–1400 cfu/m³, and ACC level 50–3000 cfu/m³. The sample size ranged from 11 to 76 with a total of 147 values across the three eligible studies.\(^{7,10,29}\) A moderately significant relationship was found for ACC \(r = 0.53\) [95% CI = 0.40–0.64], \(p < 0.001\) (Figure 5A), while TF was not significantly correlated with CO\(_2\) level \(r = −0.06\) [95% CI = −0.22–0.11], \(p = 0.51\) (Figure 5B).

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 to 70, with a total of 141 measurements of airborne microorganism concentration (ACC ranged from 378 to 3000 cfu/m³; particle mass ≤5 µg [5–61 µg/m³], and >5 µg [18.8–188 µg/m³]).\(^{10,29,31}\) 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 6A), 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 6B).

To evaluate correlations with particulate matter size, three studies provided data with a sample size ranging from 48 to 70 and a total of 198 values (ACC ranging from 50 to 650 cfu/m³, particulate matter of size ≤5 µm ranged from 8 × 10\(^3\) to 4 × 10\(^3\) particle/m³, and >5 µm from 1 × 10\(^3\) to 1.1 × 10\(^3\) particle/m³). 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 7A,B).\(^{2,8,31}\)

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 to 96 with a total of 305 values across the ten studies.\(^{7,10,11,28,32,44,45,47,48,50}\) The pooled estimated was moderately positive \(r = 0.31\) [95% CI = [0.07; 0.52]], \(p = 0.014\) (Figure 8).

4 | DISCUSSION

To 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. Although the importance of ensuring good IAQ to minimize airborne microorganism transmission is recognized,\(^{31}\) 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 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.

4.1 | Sampling approaches

There are two main approaches used in air sampling, active sampling and passive sampling. Most of the studies in the literature use active sampling (see Table 2) for the benefits that it offers since it is fast and not dependent on the local room airflow pattern. Also, active sampling provides similar results with only negligible differences regardless of the type, air flow rate and manufacturer of the device.\(^{52}\) Thus, results from papers using different types of active samplers can still be used in comparisons and can still provide useful information. Other studies use passive sampling that depends on gravity sedimentation to collect airborne microorganisms usually onto an open petri dish. 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. Airborne microorganisms that are not removed by ventilation may...
| Study                        | Country                      | Place                                         | Sampling daytime                  | Season     | Correlation ACC (cfu/m³) VS… | Temp. (°C) | RH (%) |
|-----------------------------|------------------------------|----------------------------------------------|-----------------------------------|------------|-------------------------------|------------|--------|
| Božić et al (2019)          | Bosnia and Herzegovina      | Different clinics                            | N/A                               | Feb-Mar    | r = 0.22                      | p = 0.21   | n = 35 |
| Osman et al (2018)          | Egypt                        | ICU                                          | Morning and afternoon             | Year-round | r = -0.63                     | p < 0.05   | n = 24 |
| Huang et al (2017)          | Taiwan                       | Different clinics in different hospitals     | N/A                               | Oct-Feb    |                               |            |        |
| Demirel et al (2017)        | Turkey                       | Neonatal ICU                                 | N/A                               | Year-round |                               |            |        |
| Sajjadi et al (2016)        | Iran                         | Waiting hall emergency ward                  | Morning and afternoon             | N/A        | r = 0.43                      | p < 0.01   | n = 28 |
| Mirhoseini et al (2015)     | Iran                         | ICU                                          | N/A                               | N/A        | r = 0.02                      | p > 0.05   | n = 80 |
| Tekhn et al (2013)          | Turkey                       | Burn center and clinical microbiology laboratory | N/A                               | N/A        |                               |            |        |
| Park et al (2013)           | Korea                        | Lobbies                                      | Morning, afternoon and evening    | Year-round | r = 0.43                      | p < 0.01   | n = 76 |
| Méheust et al (2013)        | France                       | Laboratory room                              | N/A                               | N/A        |                               |            |        |
| Huang et al (2013)          | Taiwan                       | Two ICU                                      | N/A                               | Aug-Oct    |                               |            |        |
| Hathway et al (2013)        | United Kingdom               | A respiratory ward                            | Morning and evening               | Aug        |                               |            |        |
| Azimi et al (2013)          | Iran                         | Nursing Stations                              | N/A                               | Jan-Apr    |                               |            |        |
| Hsu et al (2012)            | Taiwan                       | Different units                              | Morning and afternoon             | N/A        |                               |            |        |
| Augustowska and Duktiwicz (2006) | Poland                      | Wards of the pneumological department        | Morning and afternoon             | Jan-May    | r = 0.31                      | p = 0.33   | n = 12 |
| Jaffal et al (1997)         | United Arab Emirates         | Different units                              | N/A                               | N/A        |                               |            |        |

Abbreviations: ACC, airborne microbial concentration measured as aerobic colony count; n, sample size; N/A, not available; PM, particle mass concentration; PS, particulate matter of size; r, Pearson correlation coefficient; RH, relative humidity; p, Spearman’s rank correlation coefficient; Temp, temperature; TF, airborne total fungi.

aStudy conducted in hospital.

bStudy conducted in more than one hospital.
| PM ≤ 5 (µg/m³) | PM > 5 (µg/m³) | PS ≤ 5 (µm) | PS > 5 (µm) | CO₂ (ppm) | TF (cfu/m³) | Correlation TF (cfu/m³) VS... |
|----------------|----------------|-------------|-------------|-----------|-------------|-----------------------------|
|                |                |             |             |           |             | Temp. (°C)        | RH (%)   | CO₂ (ppm) |
|                |                |             |             |           |             | r = 0.11         | p = 0.54 | n = 35     |
| r = 0.53       | r = 0.43       | r = 0.39    | r = 0.39    |           |             | r = 0.30         | p = 0.08 | n = 35     |
| p < 0.01       | p < 0.01       | p < 0.05    | p < 0.05    |           |             | r = −0.29        | p < 0.05 | n = 24     |
| n = 70         | n = 70         | n = 70      | n = 70      |           |             | r = −0.43        | p < 0.05 | n = 24     |
|                |                |             |             |           |             | r = −0.29        | p < 0.05 | n = 103    |
| r = 0.53       | r = 0.43       | r = 0.39    | r = 0.39    |           |             | r = 0.22         | p < 0.01 | n = 28     |
| p < 0.01       | p < 0.01       | p < 0.05    | p < 0.05    |           |             | r = 0.29         | p < 0.05 | n = 28     |
| n = 80         | n = 80         | n = 80      | n = 80      |           |             | r = 0.72         | p < 0.01 | n = 42     |
| r = 0.59       | r = 0.01       | r = 0.59    | r = 0.30    | r = 0.12  | r = 0.32    | r = 0.01         | p = N/A  | n = 11     |
| p = N/A        | p = N/A        | p = N/A     | p = N/A     | p = 0.30  | p < 0.01   | p = N/A          | n = 11   | n = 11     |
| n = 11         | n = 11         | n = 11      | n = 80      | n = 80    | n = 80      | n = 80           | n = 80   | n = 80     |
| r = 0.29       | r = 0.35       | r = 0.58    | r = −0.19   | r = 0.17  | r = 0.02   | r = −0.19        | p < 0.01 | n = 76     |
| p = N/A        | p = N/A        | p = 0.01    | p = 0.52    | p = 0.01  | p = 0.056  | p < 0.01         | p = 0.056| n = 76     |
| n = 11         | n = 11         | n = 76      | n = 14      | n = 14    | n = 76      | r = −0.85         | p < 0.06 | n = 5      |
|                |                |             |             |           |           | r = 0.95         | p = 0.05 | n = 4      |
| r = 0.27       | r = 0.80       | r = 0.01    | r = 0.27    | r = −0.10 | r = 0.23   | r = −0.10        | p = N/A  | n = 48     |
| p = 0.6        | p = 0.05       | p = 0.01    | p = 0.6     | p = 0.78  | p = 0.52   | p = N/A          | n = 10   | n = 10     |
| n = 48         | n = 48         | n = 48      | n = 48      | n = 10    | n = 10      | r = −0.10         | p = N/A  | n = 60     |
| r = 0.12       | r = 0.08       | r = 0.44    | r = 0.29    | r = −0.12 | r = 0.29   | r = −0.12        | p = 0.35 | n = 12     |
| p = N/A        | p = N/A        | p = N/A     | p = N/A     | p = 0.71  | p = 0.35   | p = N/A          | p = N/A  | n = 12     |
| n = 60         | n = 60         | n = 60      | n = 60      | n = 12    | n = 12      | r = −0.17         | p = 0.60 | n = 12     |
| r = −0.21      | r = 0.73       | r = −0.21   | r = −0.21   | r = −0.17 | r = −0.21  | p = 0.73         | n = 5    | n = 5      |
| p < 0.01       | p < 0.01       | p < 0.01    | p < 0.01    | p < 0.01  | p < 0.01   | p = 0.73         | n = 5    | n = 5      |
| n = 60         | n = 60         | n = 60      | n = 60      | n = 60    | n = 60      | r = −0.21         | p < 0.01 | n = 5      |

Note: Correlation values are calculated for various environmental factors such as temperature (Temp.), relative humidity (RH), and CO₂ concentration (CO₂). The table includes correlation coefficients (r), p-values (p), and sample sizes (n) for different data sets from various studies, including those conducted in hospitals, clinics, and laboratories.
FIGURE 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

FIGURE 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

FIGURE 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 with airborne total fungi concentration
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/m². 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 min and duration of 5 min each over 8 h of sampling. The summary of results in Table 2 shows 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 leads to misleading conclusions as the results are too few to reflect the accurate correlation.

4.2 Sources and activity

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. Studies show high variability in measurements between different studies in different locations which may be influenced by multiple parameters as illustrated in Table 1. A small number of studies show fluctuation with time in the same location and demonstrate the complexity of interactions, with microorganisms, particles, and CO₂ 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 mold 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.

4.3 Temperature and Humidity

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 was 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 is low. This makes physical sense as most microorganisms favor 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. Another study found that there is no significant relationship between atmospheric temperature (16°C and 24°C) and survival rate of airborne Serratia marcescens, Escherichia coli, and Bacillus subtilis.

Many bacteria and fungi favor 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 regard to survival. 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. 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). Some viruses also express a U-shaped response with the lowest survival at mid-range humidity (40%-60% RH). Humidity may also have a further effect where microorganisms are released into the air through aerosolization from a liquid, with lower humidity resulting in smaller aerosols which may be suspended for longer in the environment.

Recommendations for temperature and humidity in hospitals vary by country, season, ventilation strategy, and 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. In the United States, ASHRAE recommends 21-24°C in patient rooms and also does not specify humidity control and however in clinical areas, they typically recommend 30%-60% RH. 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). 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 measure 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 affect 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.

4.4 Ventilation rate

The moderate and positive significant relationship between airborne bacteria concentration and CO₂ 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 (1) ventilation, which is the process of diluting, removing and replacing the air in a specific area naturally or mechanically, and (2) 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.\(^7,^{10,57}\)

Conversely, there was no relationship between TF concentration and $\text{CO}_2$ level. This result can be interpreted according to previous work that found people shed half the number of bacteria as fungi.\(^58\)

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.\(^26\) Studies have shown that the level of $\text{CO}_2$ level has a positive correlation with occupied rooms, room temperature, and relative humidity.\(^7,^{10,59}\) Although it is possible to estimate ventilation rates using exhaled $\text{CO}_2$ 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 United States, ASHRAE recommends 6 ACH and 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. 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.

### 4.5 Particulates

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 is 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 and 1000 s). This study result shows that there is significantly moderately positively correlation between airborne microorganisms, particle mass concentration (≤5) µg/m³, and diameter particle concentration (≤5 and >5 µm) particle/m³, 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 (eg, 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. Additionally, human occupancy has a strong link with indoor particle mass concentration. Much higher particle mass concentrations may however be associated with outdoor air pollution which would not be expected to be correlated with microorganism sources within a hospital ward. As a result, the DAG method (Figure 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 in a building.

### 4.6 Limitations

For most of the analysis, data are 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. Previous work shows that the infection status of the patients (not colonized, infected, and/or colonized) and clinical care activities are both correlated with increased concentrations of airborne microorganisms. 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, these normally occur during a sampling snapshot, and there are 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 IAQ are essential to design effective studies.

### 5 Conclusion

We have systematically reviewed studies that sampled airborne microorganisms in hospital wards and presented quantitative data with one or more IAQ parameters (temperature, relative humidity, CO₂, particle mass concentration, and particulate matter of size). We found that there are 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 (eg, operating rooms). Overall, we can conclude the following from the meta-analysis:

1. There are likely to be positive correlations between airborne bacteria and other types of microorganism, particularly fungi.
2. There are positive correlations between airborne bacteria and fungi, measured as ACC, and several IAQ parameters.
# TABLE 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)                        | Samp’Air™ Lite                          | bioMérieux, France                                | 200             | N/A                    | N/A              | 3–4                                | TSA                                           | SDA                |
| Osman et al (2018)                         | Andersen two-stage viable cascade        | Tisch Environmental Cleves, OH, USA               | 28.3            | 5                      | 141.5            | 4                                  | Nutrient agar (with cycloheximide)            | Rose-Bengal streptomycin agar                  |
| Huang et al (2017)                         | MAS-100                                  | Merck Inc., USA                                    | 100             | N/A                    | N/A              | 2                                  | TSA                                           | N/A                |
| Demirel et al (2017)                       | MAS-100                                  | Milipore bioMérieux                                | N/A             | N/A                    | 100              | 1                                  | N/A                                           | Dichloran 18% glycerol agar                    |
| Sajjadi et al (2016)                       | Air sampling pump                        | SKC                                               | 61              | 5                      | 305              | 2                                  | TSA (with nystatin)                           | SDA (with chloramphenicol antibiotic)          |
| 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)               | Passive sampling (9-cm-diameter petri dishes) | N/A                                               | N/A             | N/A                    | N/A              | 2                                  | 2% nutrient agar                              | 4% SDA              |
| Yang et al (2014)                          | Single-stage impactor                    | Standard BioStage, SKC Inc., PA, USA              | 28.3            | 2                      | 56.6             | 2                                  | TSA                                           | MEA                |
| Tekın et al (2013)                         | Air Test Omega                           | LCB, France                                       | N/A             | N/A                    | N/A              | 2                                  | plate count agar (PCA)                        | SDA                |
| Park et al (2013)                          | Anderson single-stage cascade            | N/A                                               | 28.3            | 5                      | 141.5            | 3                                  | TSA                                           | SDA (with chloramphenicol)                     |
| Méheust et al (2013)                       | Samp’Air AirIdeal AirPort MD8/BACTair    | AES Chemunex, France                              | 100             | 1–10                   | 100–1000         | 5                                  | TSA                                           | SDA                |
| 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)                       | Microbio MB2                             | Fred Parrett, UK                                  | 500             | 5                      | 2500             | 32 (Every 15 min)                  | TSA                                           | N/A                |
| Azimi et al (2013)                         | Andersen one-stage viable single-stage viable cascade | Quick Take-30, SKC, USA SKC BioStage single-stage viable cascade impacters | 28.3            | 2                      | 56.6             | N/A                                | N/A                                           | SDA                |

(Continues)
(temperature, CO₂, particulate matter of size of ≤5 and >5 µm, and particle mass concentration ≤5 µg/m³). However, the data did not demonstrate a clear correlation with relative humidity, and correlations between TF and IAQ parameters were weak.

3. There are 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.

Our conclusions lead to the following recommendations:

1. There is a need for more detailed sampling studies including air sampling (active and passive), measurement of air quality parameters, and observation of level of healthcare worker activity to understand the spatial and temporal fluctuation in microbial bioburden in hospitals.

2. Reporting of data should be quantitative as far as possible to enable comparison between studies and future meta-analysis. It is difficult to compare studies that present microbial samples in terms of percentage positive or in a semi-quantitative way. Studies that carry out statistical analysis should provide the correlation coefficient and the sample size.

3. Instead of referring to the season or geographic location of the study, seasonal factors need to be reported quantitatively in terms of temperature and relative humidity to ensure they are consistent and comparable between different locations around the world.

4. It is important that data are reported at the time that each sample is taken rather than as an average for the whole study. Studies that simply present IAQ or ventilation parameters as a mean and standard deviation across all the samples do not provide sufficient data for further analysis.

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CONFLICT OF INTEREST

None to declare.

PEER REVIEW

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| Study | Manufacturer | Airflow (l/min) | Sampling duration (min) | Total volume (l) | Interval between samples (per day) | ACC media | TF Media | TF Media |
|-------|--------------|----------------|-------------------------|------------------|------------------------------------|-----------|---------|---------|
| Hsu et al (2012) | N/A | N/A | 2 | N/A | N/A | ACC media | N/A | N/A |
| Augustowska and Dutkiewicz (2008) | Casella, London | 30 | 5 | 150 | N/A | Blood agar | SDA |
| Jaffal et al (1997) | N/A | N/A | 4 | N/A | N/A | ACC media | N/A | N/A |
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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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