Health Information and Quality Authority

An tÚdarás Um Fhaisnéis agus Cáilíocht Sláinte

Evidence summary for airborne transmission of SARS-CoV-2 via aerosols

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Key points

- Transmission of respiratory viruses is typically through three modes: contact, droplet (typically >5µm diameter) and or aerosol (typically ≤ 5µm diameter). This review concentrates on the potential airborne transmission of SARS-CoV-2 via aerosols.
- Each mode of transmission, and the degree to which it contributes to the overall spread of SARS-CoV-2, has important connotations for public health guidance. This is particularly true with regards to healthcare precautions and personal protective equipment use.
- This review included 28 studies with various designs; epidemiological case series (n=8), air sampling (n=16), and microbiological (n=4).
- Results from seven of the eight epidemiological case series included suggest aerosols may contribute to SARS-CoV-2 transmission, with enclosed environments, poor ventilation, and low temperatures noted as possible contributing factors.
- The results of the 16 air sampling studies highlight that it is likely that SARS-CoV-2 can be present in air. Three of these studies attempted to culture the virus from positive air samples, one of which was successful. Successful culture was also reported in another air sampling study published following completion of this review. These two studies, which cultured virus from air samples taken within hospitalised COVID-19 patients' rooms, provide plausibility for the clinical infectivity and viability of the virus in this form.
- Evidence from two microbiological studies indicate that SARS-CoV-2 is viable in aerosols, for up to 16 hours in one study. A further two microbiological studies highlight that virus viability is substantially reduced by simulated sunlight and extreme heat (>200°C). The controlled laboratory nature of these studies may not be reflective of real-world environments.
- The quality of evidence from the epidemiological studies was low, due to the inherent biases associated with these study designs. A formal quality appraisal tool was not identified for air sampling or microbiological studies, therefore these studies were not appraised within the context of this review. Of note, a

large number of studies included (13/28) are published as pre-prints and have not yet been formally peer-reviewed.

- Taken collectively, the results of this review indicate that, as yet, there is no conclusive evidence regarding the viability and infectivity of SARS-CoV-2 in aerosols.
    - Epidemiological studies suggest possible transmission.
    - Air sampling studies have detected viral particles with one study noting successful cultivation in a limited number of samples.
    - Microbiological studies indicate such particles may represent live virus, adding plausibility for clinical infectivity.
- In conclusion, while there is some limited, low certainty evidence that SARS-CoV-2 may transmit via aerosols, it is not known if this is restricted to specific contexts, such as in low temperature, enclosed or poorly ventilated environments. It is also uncertain what contribution aerosol transmission makes to the COVID-19 pandemic relative to other transmission modes (contact and droplet).

Evidence summary for airborne transmission of SARS-CoV-2 via aerosols

The Health Information and Quality Authority (HIQA) has developed a series of 'Evidence Summaries' to assist the Clinical Expert Advisory Group (EAG) in supporting the National Public Health Emergency Team (NPHET) in their response to COVID-19. These summaries are based on specific research questions. This review was developed to address the following research question:

> **Does airborne transmission via aerosols contribute to the spread of SARS-CoV-2?**

Background

Transmission of respiratory viruses is typically through three modes: contact, droplet and or aerosol.[1-3] Contact transmission can be direct, such as on an infected individual's hands, or indirect through the presence of virus particles on intermediate objects known as fomites.[2, 3] Droplet transmission occurs with exposure to large infectious respiratory particles containing viral material from a symptomatic individual, such as through coughing or sneezing, and typically requires close contact as the particle size denotes a relatively limited travel distance before settling to the ground or surrounding surfaces (less than one meter).[1, 3] Airborne transmission is defined as the spread of an infectious agent caused by the dissemination of aerosols (droplet nuclei).[3] Such transmission is distinct from droplets as it is based on smaller particle size, enabling a greater travel distance and the potential to remain suspended in air for prolonged periods.[2, 3] Aerosols are emitted to varying degrees and sizes depending on the activity in question such as breathing, talking, singing, and residually following coughing or sneezing.[1] The definition of aerosols is challenging, with the World Health Organization (WHO) assigning a cut-off of particles <5μm in diameter.[3] However, a dichotomous definition has been highlighted as somewhat ambiguous with little definitive support.[1] Studies of respiratory transmission have varied widely in the size definitions used, with a size definition for aerosols of <10μm frequently seen.[1, 2]

Regardless of the size definition, the principle difference between aerosol and droplet transmission is the infection risk of aerosols through airborne contamination. This has important connotations for public health decision-making for the general population and healthcare workers.[1, 2] The risk of airborne transmission, and the virulence of the respective pathogen, are important considerations that inform infection prevention and control measures including the requirement for, and type of PPE that should be worn by healthcare workers (surgical masks or respirators), and the use of face coverings by the general population.[4, 5] For instance, measles is a

highly infectious respiratory agent, which can transmit via aerosols and requires the implementation of strict airborne precautions and use of sophisticated PPE (e.g. respirators).[6] In their scientific brief, updated 9 July 2020, the WHO recommends contact and droplet precautions when caring for COVID-19 patients, with airborne precautions considered appropriate during aerosol generating procedures (AGPs) such as intubation.[3] This stance is also reflected in the guidance provided by the Health Protection Surveillance Centre[7] and Public Health England.[8] Interim US Centers for Disease Control and Prevention (CDC) guidance highlights that while the use of N95 respirators is preferred (over surgical face masks) when caring for COVID-19 patients, this form of PPE should be prioritised for situations where respiratory protection is most important and in the care of patients with pathogens requiring definite airborne precautions (for example tuberculosis, measles, and varicella zoster).[9] Hence, the determination of the risk of a respiratory pathogen to transmit via aerosols, and the associated virulence, is particularly important in the context of pandemic settings such as COVID-19 where preservation of PPE supplies and a balanced risk assessment is crucial.[4, 10]

Methods

The processes as outlined in HIQA's protocol (available here) were followed. Below is a summary of all relevant evidence for aerosol transmission of SARS-CoV-2 identified from 1 January 2020 until 27 July 2020.

Results

Twenty-eight studies were included in this review.[11-38] A summary of the included studies is provided in Tables 1, 2 and 3. Eight studies represented epidemiological case series of SARS-CoV-2 clusters or outbreaks (with one including a mechanistic analysis),[11, 14, 15, 23, 30, 33-35] 16 were air sampling studies,[12, 13, 16-19, 21-23, 25, 26, 31, 32, 36-38] and four were microbiological studies.[20, 27-29] The results of this review are presented and summarised in sections titled by study design.

Epidemiological studies

Eight studies were epidemiological case series assessing outbreaks or clusters of SARS-CoV-2 infection,[11, 14, 15, 23, 30, 33-35] with one including a mechanistic element through an onsite experiment and computer simulation.[15] Three studies related to cases in China,[11, 15, 30] two to cases in the United States,[14, 34] one to an outbreak in Germany,[35] one to the Diamond Princess cruise ship,[33] and one included a combined analysis of data from China, the United States, and Italy.[23]

Cai et al.[11] analysed a cluster of infections related to a shopping mall in Wenzhou, China. The index case was presumed to be an asymptomatic carrier with travel

association to Wuhan, China. In total, COVID-19 was diagnosed in seven employees in the same office as the index case, seven mall staff from three separate floors and 10 mall customers, alongside a number of outside contacts. From the descriptive analysis, the authors conclude that low intensity transmission appears to have occurred without prolonged close contact; that is, the virus spread by indirect transmission perhaps resulting from virus contamination of common objects, by virus aerosolisation in a confined space, or spread from asymptomatic infected persons.

Günther et al.[35] analysed a cluster of SARS-CoV-2 infections related to a shift of employees at a German meat processing plant. The presumed index case, based on sequencing and bioinformatics analyses of infections, was an asymptomatic employee who had contact with a known case from another plant where an outbreak had occurred. Excluding the index case, 29 (20.7%) of 140 employees who had worked on the same shift over three consecutive days tested positive for SARS-CoV-2 with RT-PCR. From the descriptive and statistical analysis provided, the authors highlighted that although secondary infections could have occurred through close contact, the contextual layout of the plant supports the transmission of SARS-CoV-2 within an eight metre radius of the index case's work station. Furthermore, the authors speculate that environmental conditions of the plant area, including air recirculation and low temperatures may have facilitated the spread.

Hamner et al.[14] reported a cluster of SARS-CoV-2 infections related to a choir practice of 61 people in Washington, United States. The presumed index case was symptomatic at the time of the event with active symptoms for three days prior. Excluding the index case, 52 (86.7%) of 60 attendees became ill; 32 (61.5%) of these cases were confirmed by RT-PCR testing and an additional 20 (38.5%) were considered to have probable infections. Of these cases, three were hospitalised and two died. From the descriptive analysis, the authors highlight that there were several opportunities for droplet and fomite transmission, including members sitting close to one another, sharing snacks, and stacking chairs. Furthermore, the act of singing may have contributed to transmission through emission of aerosols.

Li et al.[15] analysed the potential contribution of aerosol transmission to a cluster of SARS-CoV-2 infections across three non-associated families dining in a restaurant in Guangzhou, China. The index case was a symptomatic individual seated at a table between the other two tables of infected cases. In total, there were ten confirmed cases of COVID-19 from the three family tables. The authors provided an epidemiological analysis alongside onsite experimental and computer simulations using ethane tracer gas measurements and computational fluid dynamics. The results of the analysis indicated highest gas concentrations (simulating aerosol emission from the index case) at the primary table and the neighbouring tables of infected cases. The concentrations were reduced at neighbouring tables where no

cases were reported, and lower again at the remaining remote tables in the restaurant. The authors deduced an odds ratio of being infected with SARS-CoV-2 as being higher with higher gas concentrations (associated with a 1% increase in concentration: 1.12; 95% CI 1.01 to 1.23; p= 0.035). Although other forms of transmission may also have occurred, the authors concluded that their findings support the probability of an extended short-range aerosol spread having occurred in the poorly ventilated restaurant. A descriptive epidemiological analysis of this cluster conducted by Lu et al.,[39] which did not contain an experimental element, proposed that droplet transmission was likely to be the dominant cause and cited inconsistency with expected aerosol transmission characteristics.

Shen et al.[30] analysed two outbreaks of SARS-CoV-2 infections from two distinct events in the Zhejiang province of China. The first cluster occurred on a bus of 67 passengers travelling to a worship event with a pre-symptomatic case who became symptomatic upon returning from the event. In total, 24 passengers on the bus were infected, with distribution spread throughout the bus. No statistically significant increase in risk was found with closer proximity to the index case. No passengers on a second bus were infected, suggesting transmission occurred on the index case bus rather than at the worship event itself. In the second cluster, 30 individuals attended a training workshop across a three day period with the index case thought to be an asymptomatic female from Wuhan. In total, 15 individuals were diagnosed with COVID-19. On the bus in cluster one and the conference rooms in cluster two, central air-conditioners were on indoor re-circulation mode. The authors concluded that in both clusters, airborne transmission at least partially explains the infection rates, suggesting that closed environments with air re-circulation may play a significant role in transmission.

Bays et al.[34] analysed nosocomial outbreaks of SARS-CoV-2 in two healthcare facilities. Both index cases were admitted to hospital without suspicion of SARS-CoV-2 and hence contact, droplet or airborne precautions were not implemented. The index cases both underwent AGPs. In total, 8 (1.9%) of 421 healthcare workers who were deemed to have had exposure to the index cases tested positive with RT-PCR testing. Through an analysis of electronic medical records and structured interviews with the staff, the authors determined that close contact was the likely route of transmission. Although the secondary cases were also present for the AGPs performed, the authors highlight that these individuals had prolonged close contact with the index cases without adequate PPE, and given that there was no apparent transmission to staff or patients elsewhere on the wards, suggested that these findings are more consistent with transmission by respiratory droplets rather than airborne transmission. However, it is unclear how many of the 421 exposed healthcare workers were tested in total.

Zhang et al.[23] analysed trends in SARS-CoV-2 infections across three locations; Wuhan, New York, and Italy. However, it must be noted that the findings of this study have been brought into wide disrepute with concerns about the analysis used and the conclusions drawn, with some calling for clarification or retraction.[40, 41] The primary outcome, using a linear model of analysis, was that cases of infection were possibly avoided due to the wearing of face coverings, with 78,000 fewer infections in Italy and over 66,000 fewer infections in New York City when face coverings were mandated. The authors conclude that their findings support the hypothesis that face coverings reduce aerosol transmission of SARS-CoV-2 infection.

Almilaji et al.[33] analysed the apparent contribution of cabin occupancy to infection rates aboard the Diamond Princess cruise ship during the quarantine period implemented on the ship following an outbreak of SARS-CoV-2. It should be noted that this study was deemed to be of particularly low quality in the context of this review. In total, 619 cases were confirmed on the cruise ship of which 163 cases were recorded as having symptom onset dates during the quarantine period; details of 115 cases were assessed by the authors. Using count data from published reports, the authors report that symptomatic infection rate during the quarantine period in cabins with previously confirmed cases was not significantly higher than that in cabins without previously confirmed cases. The authors concluded that although other forms of transmission were not investigated and cannot be discounted, their findings suggest that airborne transmission between cabins may have played a role in the spread of SARS-CoV-2 during the quarantine period.

Air sampling studies

Sixteen studies included air sampling for the detection of SARS-CoV-2 viral RNA.[12, 13, 16-19, 21-23, 25, 26, 31, 32, 36-38] Six of the studies were conducted in China,[13, 16, 17, 24, 32, 36] three in Singapore,[18, 21, 26] two in the United States,[19, 38] and one each in Hong Kong,[31] Iran,[12] Italy,[37] Japan,[22] and the United Kingdom.[25] The studies were largely conducted in hospital settings including clinical and non-clinical areas with known COVID-19 patients in the vicinity;[12, 13, 16-19, 21, 24-26, 31, 32, 36-38] one study was conducted on a cruise ship which had experienced an outbreak of SARS-CoV-2.[22] Three studies analysed the exhaled breath condensate of COVID-19 confirmed patients for SARS-CoV-2 RNA.[17, 24, 31] Three studies attempted to culture virus from positive samples.[19, 25, 38]

Detection of SARS-CoV-2 RNA in air samples was reported by ten studies.[13, 16, 17, 19, 25, 26, 32, 36-38] Guo et al.[13] reported positive detection in 14/40 (35%) of air samples from an intensive care unit, including samples taken near air outlets, within patient rooms and in an office area, with detection from general wards in 2/16 (12.5%) of samples which were all in close proximity to COVID-19 patients. Similarly, on

sampling patient rooms with sampling devices in relatively close proximity to the patients' beds, Chia et al.[26] noted detection of SARS-CoV-2 in two of three patient rooms sampled (66.7%); concentrations ranged from 1.84 x 10^3 to 3.38 x 10^3 and particles identified in sizes including 1-4 µm and >4 µm. Liu et al.[16] reported positive detection in a number of samples from patient areas (range 0-19 copies m^{-3}), medical staff areas (range 0-21 copies m^{-3}), and public areas (range 0-11 copies m^{-3}). Zhou et al.[25] detected SARS-CoV-2 RNA in 14/31 (38.7%) of air samples with detection from all eight areas analysed, including both clinical and non-clinical areas. Of 32 samples assessed, Santarpia et al.[19] reported 63.2% of in-room and 58.3% of outside room air samples within a ward and quarantine unit were positive for the detection of SARS-CoV-2 RNA. An additional study by the same authors[38] noted detection of SARS-CoV-2 RNA in all particle sizes (<1 µm, 1-4 µm and >4.1 µm) from 18 air samples collected from the end of the patient beds in the rooms of six COVID-19 cases. Lei et al.[36] reported detection of SARS-CoV-2 from one air sample taken within an ICU and in a further three samples taken from an isolation ward (two from a bathroom and one from the ward itself). However, it was unclear how many samples were collected in total. Razzini et al.[37] noted detection of SARS-CoV-2 RNA in an ICU and in patient corridors in 20 (54.1%) out of 37 samples. From a range of sampling sites within a hospital and hotel quarantine facility, Ma et al.[17] noted just one positive detection of SARS-CoV-2 RNA from an unventilated hotel quarantine bathroom, while Jiang et al.[32] reported one positive air sample (3.57%, 1/28) in their study with the sample taken from a ward housing an intensive care COVID-19 patient who had undergone tracheal intubation the day prior.

Five studies did not detect SARS-CoV-2 RNA from air samples.[12, 18, 21, 22, 24] Sampling sites within these studies included intensive care wards,[12] inside and outside patient rooms,[18, 21] various locations within COVID-19 wards,[24] and inside and outside cabins of cases on a cruise ship which had been vacated 1-17 days prior to sampling.[22] Two of the authors note that dilution by air exchange or general disinfection may account for the lack of detection within air samples,[18, 24] and two studies noted SARS-CoV-2 confirmed cases were not present in the area for at least 24 hours prior to sampling.[21, 22]

Three studies presented results on the detection of SARS-CoV-2 RNA in the exhaled breath condensate of confirmed cases.[17, 24, 31] Zhou et al.[24] noted detection in 2/9 (22%) samples collected from recovering COVID-19 patients who were at least 14 days since symptom onset. Ma et al.[17] reported a detection rate of 5/30 (16.7%) from exhaled breath condensate samples taken from COVID-19 patients within 14 days of symptom onset. Cheng et al.[31] noted no positive detection of SARS-CoV-2 in the exhaled breath of six COVID-19 patients, with and without the use of surgical masks, (median of 3.5 days since symptom onset), with the authors concluding that results indicate the airborne route is not the predominant mode of transmission.

Three studies attempted to conduct virus culturing on positive samples.[19, 25, 38] Zhou et al.[25] reported no virus was successfully cultured from the 14 positive air samples within their study. Santarpia et al.[19] highlighted that although low concentration levels of the virus in the recovered air samples resulted in unsuccessful cultivation, the results from one sample indicated some evidence of the presence of a replication competent virus.[19] However, an additional study from the same authors,[38] did note statistically significant viral growth (defined as rRT-PCR samples in which a significant increase in RNA was detected in the supernatant) in three of 18 positive samples, all of which were <1 µm particle size, while two further samples of 1-4 µm particle size demonstrated viral growth but did not reach statistical significance. Supplementary western blot and transmission electron microscopy analysis of these samples also showed evidence of viral proteins and intact virions in a number of cultures.

Microbiological studies

Four microbiological studies were included within this review.[20, 27-29] All were conducted in controlled laboratory conditions in the US with two studies investigating the persistence of the SARS-CoV-2 virus in aerosolised particles,[20, 29] and two analysing the effect of varying environmental conditions on the viability of the virus.[27, 28]

A study by van Doremalen et al.[20] investigated the persistence of SARS-CoV-2 viability through the generation of aerosols replicating those produced by the upper and lower respiratory tract of infected humans in a controlled laboratory experiment. The authors noted the SARS-CoV-2 virus remained viable in aerosols throughout the duration of the three-hour experiment (reduction in infectious titre from $10^{3.5}$ to $10^{2.7}$ $TCID_{50}$ per litre of air) and presented a median half-life estimate of 1.1 hours (95% credible interval 0.64 to 2.64), highlighting a plausibility for aerosol transmission of the virus. The authors noted similar viability results for SARS-CoV-1 and SARS-CoV-2 when the two viruses were directly compared. Fears et al.[29] analysed the short- and long- term efficiency of the SARS-CoV-2 virus in aerosols. The authors noted the short-term dynamic aerosol efficiency of SARS-CoV-2 surpassed those of SARS-CoV-1 and MERS, while longer term analysis indicated detection of SARS-CoV-2 in aerosols at five time points of a singular experiment (up to 16 hours) with minimal decreases in concentration measured in viral genome copies. Examination with electron microscopy highlighted that virus particles aged for 10 minutes or 16 hours were similar in shape and general appearance to those examined in samples collected before aerosolisation. The authors noted that these collective results potentially indicate retained infectivity and virus integrity of SARS-CoV-2 for up to 16 hours in aerosols.

In terms of the effect of environmental conditions on SARS-CoV-2 in aerosols, Schuit et al.[27] investigated the effect of varying levels of humidity and simulated sunlight on the persistence of the SARS-CoV-2 virus. Within controlled laboratory experiments, the authors noted that variations in relative humidity alone did not affect the decay rate. However, simulated sunlight inactivated the virus in aerosols in both suspension matrices tested, with half-lives of less than 6 minutes and 90% of the virus inactivated in less than 20 minutes for all simulated sunlight levels investigated. With regards to temperature, Yu et al.[28] noted a 2.7-fold log reduction $TCID_{50}$ (estimated 99.8% viral load reduction) in SARS-CoV-2 suspended aerosols with the use of a novel nickel air filter heated to approximately 200°C.

Study quality and quality of the evidence

The quality of evidence from the epidemiological studies was low, due to the inherent biases associated with these retrospective observational study designs. Where applicable, the majority of studies provided sufficient detail of case descriptions, context and detection of outcome. Of the four studies employing statistical techniques, two were deemed to be appropriate.[15, 30] The use of linear regression in the study by Zhang et al.[23] was deemed inappropriate in the context of their analyses, with additional critique in terms of the lack of a control population and the exclusion of a lag time between infection and reported cases. Further concerns were also raised about the causative conclusions drawn given the associative nature of the analyses in this study. Additionally, the conclusions drawn from the count data analysis conducted by Almilaji et al.[33] raised considerable concerns given that only a subset of the available data was used and potential confounders were not accounted for.

A formal quality appraisal tool was not identified for air sampling or microbiological studies, therefore these studies have not been formally appraised within the context of this review. The majority of air sampling studies provided a reasonable degree of information relating to the methodology employed, including collection methods, timing of collection and sampling sites. However, the quality of the methodologies employed were not appraised. The use of PCR testing and the choice of gene targets were typically well reported, however the thresholds for detection were inconsistent across studies and were unclear in a number of the studies. Similarly, the microbiological studies provided a large level of detail regarding the methodology employed and the conditions assessed. Based on the data provided, it was not possible to ascertain if the conditions in the studies reflect real-world environments.

Thirteen out of the 28 studies (46%) included in this review are published as pre-prints at the time of writing, so have not yet been formally peer-reviewed raising

additional concerns about overall quality and the potential for results to change prior to formal publication.

Discussion and conclusion

The results of this review present a collection of evidence regarding the airborne transmission of SARS-CoV-2 via aerosols from three study types: epidemiological, air sampling, and microbiological. The collective results from the epidemiological analyses of SARS-CoV-2 clusters or outbreaks suggests that aerosol transmission may possibly play a role, amongst other transmission routes, but the confidence in the possibility of this role or its relative contribution is largely uncertain. Air sampling studies provide evidence that SARS-CoV-2 RNA is detectable in a proportion of samples collected in clinical and non-clinical areas. However, the viability and infectivity of the virus in these environments was poorly investigated. Of three studies that attempted to culture virus from positive air samples, only one was successful in a limited number of samples. Evidence from two microbiological studies suggest viability of the virus in aerosols with plausibility for transmission, however given the controlled laboratory nature of these studies, it is unclear if this translates to real-world environments.

Low quality evidence from seven out of eight epidemiological studies suggest possible transmission in retrospective analyses, however these studies are limited in the data that they can provide and are at an inherently high risk of bias. A novel mathematical model applied to two of the described clusters (restaurant described by Liu et al. and choir described by Hamner et al.) by Buonanno et al.[42] further proposes theoretical evidence for aerosol transmission being a reasonable cause of the high number of infections seen in these clusters. However, there is substantial uncertainty regarding both the potential for aerosol transmission and its relative contribution to the spread of the virus. The majority of studies in this review acknowledged that aerosols may play a contributory, but not an exclusive role. Kutter et al.[2] and Gralton et al.[1] highlight that modes of transmission of respiratory viruses are unlikely to be mutually exclusive, with the three forms likely contributing in varying degrees depending on the virus in question. The significant contribution of aerosol transmission is well-recognised for certain bacteria (such as Mycobacterium tuberculosis) and viruses (such as measles and varicella-zoster);[4] however, the role of aerosols, and their relative contribution to the transmission of other respiratory viruses such as influenza, SARS-CoV-1, MERS-CoV, and rhinovirus is contentious and widely debated.[2, 43, 44] This uncertainty is reflected in clinical guidelines for the care of individuals with respiratory viruses. These guidelines frequently include a range of precautionary measures, particularly during the conduct of aerosol generating procedures.[2] This uncertainty has been the source of controversial debate regarding face mask use during the COVID-19 pandemic. A recent HIQA evidence summary on the use of facemasks in the community

concluded that there was very limited, low quality evidence that face masks may reduce transmission of SARS-CoV-2 in the community, with plausibility of source control in preventing transmission via droplets and aerosols from pre-symptomatic and asymptomatic COVID-19 cases.[45] It should be further noted that the methodology used in the present review sought to retrieve evidence relating explicitly to aerosol transmission. While it could be inferred that epidemiological studies which concluded that droplet or contact transmission were dominant modes did not believe aerosols played a significant role, these studies were not eligible for inclusion unless the contribution of aerosols was explicitly assessed.

The results of the air sampling studies and evidence under laboratory conditions of sustained detection from the microbiological studies within this review indicate that SARS-CoV-2 transmission via aerosols is possible. However, the detection of the virus in the air through PCR assays merely indicates presence and does not provide information regarding viability or infection risk.[3] Only three studies within this review attempted to culture the virus from positive PCR detected air samples,[19, 25, 38], with one noting successful cultivation in a limited number of samples.[38] An additional study conducted by Lednicky et al.,[46] published since completion of this evidence summary, has further shown virus cultivation from air samples taken in the hospital room of two COVID-19 patients; with samples were taken at a distance of at least two metres from the patients. This paper is also a preprint which has not yet been formally peer-reviewed. The use of supplementary virus culturing provides greater insight to the viability of the virus overall, with positive cultures providing plausible evidence of clinical risk. However, it is noted that such studies are notoriously challenging to complete and results may be impacted by other parameters or methodologies used.[47, 48] Therefore, a failure to culture SARS-CoV-2 in these studies may reflect the challenges in these study types or accurately indicate low pathogen levels.

A further important consideration in the transmissibility of respiratory viruses is the contribution and effect of environmental factors such as relative humidity, temperature and radiation.[48] Two microbiological studies within controlled laboratory environments further highlighted degradation of the virus with exposure to varying degrees of simulated sunlight,[27] and when subjected to high temperatures through a novel nickel filter;[28] no substantial effect was noted for varying levels of relative humidity.[27] Given the laboratory nature of these studies translation to real-world environments is uncertain. A number of the epidemiological studies within this review noted that poor ventilation or air recirculation may have contributed to the spread of the virus, with another also citing low temperatures in a factory setting as a potential contributing factor.[11, 15, 30, 35] The potential for this contextual transmission of SARS-CoV-2 has been acknowledged by the World Health Organization,[3] and within theoretical assessments of the potential role of airborne

transmission to the COVID-19 pandemic.[49, 50] However, both have emphasised that, should airborne transmission occur, it is likely to be opportunistic, with such environments playing a facilitator role,[49, 50] while noting also that the reproduction rate for SARS-CoV-2 appears substantially lower than other established airborne viruses such as measles,[49] which is often cited as being between 12 and 18.[51]

Investigations of the relative contribution that each mode of transmission makes to the spread of specific respiratory pathogens is a particularly challenging area of research.[2, 4] Study designs which involve direct human transmission are naturally ethically flawed; in their absence a myriad of experimental designs are employed, each with clear advantages and disadvantages in their ability to definitively answer such a research question.[2] Therefore, conclusions about the likely modes of transmission, and their relative contribution, are typically made with consideration of a broad and multidimensional evidence-base. The form of evidence-base typically takes a considerable degree of time to mature, and often draws conclusions of mixed transmission routes, with different routes predominating depending on specific contexts such as environmental setting or exposure time.[4] Such an evidence-base is currently lacking in terms of the potential for the spread of SARS-CoV-2 via aerosol transmission, but more robust conclusions may be drawn as additional studies are published in this rapidly emerging area. In the context of the ongoing COVID-19 pandemic, and citing some of the limited evidence-base reported here, certain scientists have suggested that the precautionary principle should therefore apply.[52]

In conclusion, the results of this review present a collection of evidence regarding the potential for airborne transmission of SARS-CoV-2 via aerosols from three study types: epidemiological, air sampling, and microbiological. Limited, low certainty evidence from a small number of retrospective epidemiological studies suggest possible aerosol transmission of SARS-CoV-2. Furthermore, results from air sampling and microbiological studies add plausibility to the potential for SARS-CoV-2 to transmit via aerosols, with some evidence of clinical infectivity. Overall, while there is some evidence to suggest a potential for SARS-CoV-2 to transmit via aerosols, it is uncertain what contribution it makes relative to other transmission modes (contact and droplet) to the COVID-19 pandemic, and whether such transmission is context dependent, for example, in poorly ventilated or enclosed environments. Additional well-conducted and high-quality studies, across the spectrum of experimental designs, would provide greater insight into this research question in this rapidly evolving research area.

Table 1. Summary of epidemiological studies

Author Country Study design Status: DOI	Setting Clinical characteristics	Cluster description	Analysis method	Results
Almilaji 2020 **Analysis of Diamond Princess cruise ship** **Epidemiological case series** **Preprint:** https://doi.org/10.1101/2020.07.08.20148775	**Setting:** Outbreak on the Diamond Princess cruise ship **Clinical characteristics:** NR **SARS-CoV-2 detection:** All cases laboratory confirmed	▪ Analysis of count data from all recorded positive cases from on board testing clinic ▪ 619 COVID-19 positive cases were confirmed ▪ Among these, there were 163 cases with recorded symptom onset dates during the quarantine period, of which data from 115 cases was included in analysis	▪ Count data of all confirmed cases analysed with details of cabin occupancy during quarantine period of ship outbreak ▪ Symptomatic infection rates during the quarantine period in cabins with previous confirmed cases compared to those in cabins without previous confirmed cases	**Authors' conclusions:** Symptomatic infection rate during the quarantine period in cabins with previously confirmed cases is not significantly higher than that in cabins without previously confirmed cases. Though not discounting other important transmission modes such as close-contact droplets and fomites, in this study, only the airborne transmission mode was considered to explain the infection with COVID-19 in passengers' cabins during the quarantine period.
Bays 2020 **United States** **Epidemiological case series** **Published:** 10.1017/ice.2020.321	**Setting:** Nosocomial outbreak involving healthcare workers in a community hospital and a university medical centre **Clinical characteristics:** Two index cases admitted without initial suspicion of COVID-19. **SARS-CoV-2 detection:** All cases detected with RT-PCR, however unclear how many exposed individuals were tested.	**Index patient:** Two index cases without contact or droplet precautions in place; both patients underwent several aerosol generating procedures (AGPs). **Confirmed cases:** 8/421 exposed healthcare workers confirmed as positive however unclear how many exposed individuals were tested. **Estimated distances from index case:** All 8	Descriptive analysis of the electronic medical record tracing in combination with structured interviews.	**Authors' conclusions:** All confirmed cases had prolonged direct contact with the patient including during AGPs without adequate personal protective equipment. Authors conclude no evidence of airborne transmission as transmission occurred exclusively amongst staff that were at the patient's bedside without contact and droplet PPE (although also present for AGPs). There was no apparent transmission to staff or patients elsewhere suggesting these findings are more consistent with transmission by respiratory droplets rather than airborne transmission. These observations suggest that, at least in a healthcare setting, a majority of SARS-CoV-2

		staff cases, had close contact with the index patients without sufficient personal protective equipment		transmission is likely to take place during close contact with infected patients through respiratory droplets, rather than by long-distance airborne transmission.
Cai 2020 **China** **Epidemiological case series** **Published as research letter:** https://dx.doi.org/10.3201/eid2606.200412	**Setting:** Cluster of SARS-CoV-2 infections in a shopping mall in China **Clinical characteristics:** Presumed index case thought to be asymptomatic carrier **SARS-CoV-2 detection:** All cases and contacts detected with RT-PCR	**Index patient:** Presumed to be a patient who had associated travel to Wuhan (Patient A). Patient A thought to be an asymptomatic carrier **Confirmed cases:** COVID-19 was diagnosed in 7 employees in the same office, 7 mall staff from 3 separate floors and in 10 mall customers. **Estimated distances from index case:** Except for those who had been on the same floor as the office cases, all other case-patients denied direct close contact with other case-patients **Contact tracing:** Close contacts associated with the mall were traced, and COVID-19 was confirmed for 11 individuals.	Descriptive analysis of case characteristics and behaviours.	**Authors' conclusions:** Findings appear to indicate that low intensity transmission occurred without prolonged close contact in this mall; that is, the virus spread by indirect transmission perhaps resulting from virus contamination of common objects, virus aerosolisation in a confined space, or spread from asymptomatic infected persons.
Gunther 2020 **Germany** **Epidemiological case series** **Preprint:** https://www.ssrn.com/abstract=3654517	**Setting:** Meat processing plant. Prior to events described German authorities sanctioned SARS-CoV-2 PCR-based series testing of the entire staff of such plants **Clinical characteristics:**	**Index patient:** Asymptomatic case who had contact with known case from an outbreak in another meat processing plant **Confirmed cases:**	Descriptive analysis of case characteristics, behaviours, living quarters and work station proximity to index case Sequencing and bioinformatic analysis	**Authors' conclusions:** ▪ The probability for spatial overrepresentation of positive cases was significant and reaches a maximum (p-val 2.33E-05) within a radius of 8 m from the work station of the index case. ▪ While some secondary infections may have occurred within apartments,

	Index case had no symptoms **SARS-CoV-2 detection:** All cases detected with RT-PCR.	▪ 29/140 employees on shift with index cases tested positive **Estimated distances from index case:** Most employees on shift worked at fixed positions in a conveyor-belt processing line occupying an elongated area approximately 32m long and 8.5 m wide. Eight air conditioning units in proximal area of room. Index case situated at a fixed work position within proximal area.	also undertaken	bedrooms or carpools, collective data strongly suggest that the majority of transmissions occurred within the beef processing facility. ▪ Transmissions occurred in a confined area of a meat processing plant in which air is constantly recirculated and cooled to 10°C. Index case transmitted the virus to co-workers in a radius of more than 8m during work-shifts on 3 consecutive days. ▪ Findings suggest that the facilities' environmental conditions, including low temperature, low air exchange rates, and constant air re-circulation, together with relatively close distance between workers and demanding physical work, created an unfavourable mix of factors promoting efficient aerosol transmission of SARS-CoV-2 particles.
Hamner 2020^ **United States** **Epidemiological case series** **Published:** http://dx.doi.org/10.15585/mmwr.mm6919e6	**Setting:** Cluster of SARS-CoV-2 infections linked to a 2.5 hour choir practice in Washington. Sixty-one people in attendance. **Clinical characteristics:** Presumed index case symptomatic at March 10 choir practice (likely point-source exposure event, given multiple practices) **Population characteristics:** Among the 61 choir members who attended the March 10 practice, the median age was 69 years (range=31–83 years); 84% were women. **SARS-CoV-2 detection:** All cases detected with RT-PCR with additional probable	**Index patient:** Presumed to be symptomatic choir member who had symptoms for 3 days prior **Confirmed cases:** Of the 61 attendees 32 were confirmed by RT-PCR testing and 20 were considered to have probable infections Median age of those who became ill was 69 years, 85% of cases occurred in women. **Estimated distances from index case:** Chairs were arranged in 6 rows	Descriptive analysis of case characteristics and behaviours	**Authors' conclusions:** Several opportunities for droplet and fomite transmission, including members sitting close to one another, sharing snacks, and stacking chairs at the end of the practice. The act of singing, itself, might have contributed to transmission through emission of aerosols, which is affected by loudness of vocalisation

	infections noted	of 20 chairs each, spaced 6–10 inches apart with a centre aisle dividing left and right stages. No further detail provided due to patient confidentiality		
Li 2020*^ **China** **Epidemiological case series/ Mechanistic study** **Preprint:** https://doi.org/10.1101/2020.04.16.20067728	**Setting:** Cluster of SARS-CoV-2 infections in three non-associated families dining in a restaurant in China on 24 January 2020 **Clinical characteristics:** Index patient symptomatic on day of restaurant visit **SARS-CoV-2 detection:** All cases detected with RT-PCR	**Index patient:** Family member of one table in the middle of the three tables. **Confirmed cases:** 10 cases confirmed from restaurant including index case. Cases from three non-associated families seated at three separate tables. **Estimated distances from index case:** 1 metre to 4.6 metres **Contact tracing:** 193 patrons in the restaurant who were not infected.	**Video analysis:** No significant close contact between the 3 families in the elevator or restroom. **Simulation:** Tracer gas measurements and computational fluid dynamics (CFD) simulations were used to predict the spread of fine droplets exhaled by the index patient and the detailed airflow pattern in the restaurant with consideration for environmental factors. The same CFD model was used in 2 SARS outbreaks in Hong Kong in 2003. Ethane gas through an 8-mm inner diameter pipe at a speed of 1.5 m/s at 32–34 °C to mimic index patient speaking.	**Simulation:** Average ethane concentrations were highest at index and neighbouring tables of infected cases (1.00, 0.92, and 0.96 respectively) while the concentrations were 0.86 and 0.73 at non-infected neighbouring tables respectively, and 0.55–0.70 at the other remote tables. Higher concentrations created by an enveloped contamination zone due to air-conditioning units. **Regression model:** Odds ratio of being infected with SARS-CoV-2 higher with higher gas concentrations (associated with a 1% increase in concentration: 1.115; 95% CI: 1.008–1.233; $p = 0.035$). **Author conclusions:** Epidemiologic analysis, onsite experimental tracer measurements, and airflow simulations support the probability of an extended short-range aerosol spread of the SARS-CoV-2 having occurred in the poorly ventilated and crowded restaurant.
Shen 2020 **China** **Epidemiological case series** **Preprint:** 10.13140/RG.2.2.36685.38881	**Setting:** Two clusters of SARS-CoV-2 infections in Zhejiang province of China. ▪ Cluster one - 126 passengers on two buses 100-minute round trip to attend a worship event (150 minutes' duration).	**Index patient:** ▪ Cluster one- female on one of two buses. **Confirmed cases:** ▪ Cluster one - On the 1st bus there 67 people and a driver, of whom, 24	Descriptive analysis of case characteristics and behaviours.	▪ Cluster one- Passengers on bus with index case had a 41.5 (95% confidence interval [CI]: 2.6–669.5) times higher risk of being diagnosed with COVID-19 compared with those on the 2nd bus. Passengers sitting closer to the index case on the exposed bus did not have statistically higher risk of COVID-19

	▪ Cluster two - 30 individuals attending a 3-day workshop in conference rooms. **Clinical characteristics:** ▪ Cluster one- index patient pre-symptomatic during the bus trip but started to have cough, chills, and myalgia on the evening after returning from the temple ▪ Cluster two- index patient from Wuhan reported no fever at workshop however developed symptoms after (mild fever). **SARS-CoV-2 detection:** All cases detected with RT-PCR	passengers (including the index patient) were diagnosed. No individuals on the 2nd bus were diagnosed. ▪ Cluster two - total of 15 trainees who attended the workshop, including the index patient, were diagnosed. **Estimated distances from index case:** ▪ Cluster one- Index patient sat in the middle seat on the 3-seat side of the 8th row. Besides the passengers sitting close to the index patient, the seats of other cases were scattered in the bus. 100 minute round trip. ▪ Cluster two- Individuals voluntarily took seats before each of the 3 day sessions and could not recall their seat orders for all sessions. **Contact tracing:** ▪ Cluster one at the worship event, 7 were subsequently diagnosed with		compared with those sitting further away. ▪ Cluster two- overall attack rate was 48.3% (95% CI, 31.4-65.6). ▪ Central air-conditioners in indoor re-circulation mode were in use on both buses in Cluster one and in the conference rooms in Cluster two. **Authors' conclusions:** In both clusters airborne transmission at least partially explains the extraordinary attack rate seen suggesting that in closed environments with air re-circulation, COVID-19 is a highly transmissible pathogen.

		COVID-19, and all of them described being in close contact with the index case during the event. Family members of index case also diagnosed.		
Zhang 2020 **China (Wuhan), United States (New York), Italy** **Epidemiological case series** **Published:** **https://doi.org/10.1073/pnas.2009637117**	**Setting:** Epidemiological analysis of trends in three locations: Wuhan, China; Italy; and New York City. Data analysed between 23 January and 9 May 2020. **Clinical characteristics:** Case numbers across whole populations analysed **SARS-CoV-2 detection:** Presumed detection by RT-PCR as this was the standard method of case detection in these locations.	▪ Population-based analysis of the total number of cases in each location over time. ▪ Data for accumulative confirmed infections in Wuhan, Italy and NYC were taken from the reports by Wuhan Municipal Health Commission, European CDC and NYC government.	▪ Primary method of analysis was linear regression. ▪ Projection of the pandemic trend without implementing face covering in Italy and NYC was performed by fitting a straight line between the infection number and date. ▪ The slope and the reported infection number were used for the projections; avoided infections due the face covering was the difference between the projected and reported values on May 9, 2020.	▪ The primary result from the linear model was the avoided cases of infection due to face coverings. ▪ Authors estimate 78,000 infections avoided in Italy from 6 April to 9 May and over 66,000 infections avoided in New York City from 17 April to 9 May with face coverings. **Authors' conclusion:** Face coverings reduced aerosol transmission

*Additional epidemiological analysis conducted by Lu et al.[39] hypothesise droplet transmission as being the dominant cause

^Mathematical model applied by Buonanno et al.[42] highlights airborne transmission plausible to rationalise high infection rates

Table 2. Summary of air sampling studies

Author Country Study design Status: DOI	Setting Patient demographics Clinical characteristics	Sampling method for aerosol detection	Test parameters	Primary outcome results
Cheng 2020 **Hong Kong** **Air sampling study (Exhaled air)** **Published:** 10.1017/ice.2020.282	**Setting:** Airborne infection isolation rooms of 6 SARS-CoV-2 confirmed patients in a single hospital. **Patient demographics:** 4 males and 2 females, age range 15-62 years. **Clinical characteristics:** 1 asymptomatic and 5 symptomatic COVID-19 patients.	**Collection:** Exhaled air: Sartorius MD8 airscan sampling device 1,000L of air at a rate of 50 L/minute with and without wearing surgical masks. **Sampling site(s):** Perpendicularly positioned at a distance of 10 cm from the patients chin. Patients were placed under a shelter using an umbrella surrounding with a plastic curtain in order to reduce the turbulence of air flow inside the shelter. **Number of samples:** 12 (with and without wearing surgical masks) **Environmental conditions:** NR **Timing of sample collection:** Range of days since symptom onset (nil, 3, 3, 4, 4, 11)	**Test:** RT-PCR **Threshold(s):** Ct values <28 considered positive test. **Gene Target(s):** RdRP **Controls:** 4 patients were asked to sneeze and spit saliva droplets directly onto the gelatin filters used in the air sampler. The saliva droplets directly spitted on gelatin filters were all positive for SARS-CoV-2 RNA.	**SARS-CoV-2 RNA detection:** No positive detection from exhaled air samples
Chia 2020 **Singapore** **Air sampling study** **Published:** https://doi.org/10.1038/s41467-020-16670-2	**Setting:** Rooms of three SARS-CoV-2 confirmed patients in a single hospital. **Patient demographics:** NR **Clinical characteristics:** Two patients symptomatic, one patient asymptomatic	**Collection:** 6 NIOSH BC 251 bioaerosol samplers flow-rate of 3.5 L/min and for 4 hours. **Sampling site(s):** Various locations in patient rooms (all within 2.1	**Test:** RT-PCR **Threshold(s):** NR however sample with Ct value of 33.22 considered negative. **Gene Target(s):** E and ORF	**SARS-CoV-2 RNA detection:** ▪ Positive detection in samples from 2/3 patient rooms (66.7%). ▪ Particle sizes >4 μm and 1–4 μm in diameter. ▪ Concentrations in air ranged from 1.84 x 103 to 3.38 x 103 RNA.

		metres, some under 1 metre) **Number of samples:** 6 **Environmental conditions:** Rooms had 12 air changes per hour, an average temperature of 23 °C, relative humidity of 53–59%, and exhaust flow of 579.6m^3/h **Timing of sample collection:** 2 patients day 5 of illness, 1 patient day 9 of illness		▪ No positive detection from patient room who was day 9 of illness.
Faridi 2020 **Iran** **Air sampling study** **Published:** https://doi.org/10.1016/j.scitotenv.2020.138401	**Setting:** Intensive care wards with confirmed COVID-19 patients (n=44) in a single hospital **Patient demographics:** NR **Clinical characteristics:** Patients with severe and critical symptoms. 22 mechanically ventilated.	**Collection:** Impinger technique (SKC Inc.) with a flow rate equal to 1.5 L min^{-1} **Sampling site(s):** 1.5 to 1.8 m from the floor and approximately 2 to 5 m away from the patients' beds. **Number of samples:** 10 **Environmental conditions:** Details of temperature, ventilation, relative humidity, carbon dioxide levels, and particle concentration provided for each sample. **Timing of sample collection:** NR	**Test:** RT-PCR **Threshold(s):** Ct values >38 were considered negative results **Gene Target(s):** RdRp and E	**SARS-CoV-2 RNA detection:** No positive detection from air sampling.
Guo 2020 **China** **Air sampling study** **Published:**	**Setting:** Intensive care unit and a general COVID-19 ward at a single hospital.	**Collection:** SASS 2300 Wetted Wall Cyclone Sampler at 300 L/min for 30 minutes. **Sampling site(s):** 3 sites-	**Test:** qRT-PCR **Threshold(s):** Ct values> 40 considered negative **Gene Target(s):** ORF and	**SARS-CoV-2 RNA detection:** ▪ Intensive care unit- 35% (14/40) of samples. o Near air outlets- 35.7% (5/14)

https://doi.org/10.3201/eid2607.200885	**Patient demographics:** NR **Clinical characteristics:** NR	Close proximity to air outlets, patients' rooms, and the doctors' office area **Number of samples:** ▪ Air in the isolation ward of the intensive care unit- 12 air supplies and 16 air discharges per hour. ▪ General ward- 8 air supplies and 12 air discharges per hour. **Environmental Conditions:** NR	N	○ Patients' rooms- 44.4% (8/18) ○ Doctor's office area- 12.5% (1/8) ▪ General ward- 12.5% (2/16) of samples ○ Only site near patient tested positive
Jiang 2020 **China** **Air sampling study** **Preprint:** https://doi.org/10.1101/2020.02.25.20028043.	**Setting:** Ward and intensive care unit of single hospital **Patient demographics:** NR **Clinical characteristics:** 15 suspected cases and 1 confirmed intensive care unit case	**Collection:** Microbial air sampler (MAS-100 ECO) at 100 litres/min **Sampling site(s):** 10 clinical areas **Number of samples:** 28 **Environmental Conditions:** NR	**Test:** RT-PCR **Threshold(s):** Ct value ≤40 considered positive test **Gene Target(s):** ORF1ab and N	**SARS-CoV-2 RNA detection:** ▪ 3.57% (1/28) of air samples. Sample from isolation ward with an intensive care unit case who had undergone tracheal intubation the day before sample collection.
Lei 2020 **China** **Air sampling study** **Published:** https://onlinelibrary.wiley.com/doi/full/10.1111/irv.12783	**Setting:** Intensive care unit and isolation ward of single hospital dedicated to treatment of severe and critical COVID-19 patients. **Patient demographics:** Collected from 5 patient rooms; four males and one female, age range 26-82 years. **Clinical characteristics:** Three severe illness, one critical and one mild. Three of the four patients were receiving mechanical ventilation and had aerosol generating procedures during sampling times.	**Collection:** NIOSH air sampler (4 hours at a flow rate of 3.5 L/min into 3 size fractions: >4 μm, 1-4 μm, and <1 μm) and a cyclonic aerosol particle liquid concentrator (model W-15, Beijing DingBlue Technology, flow rate of 14 L/min for 30 minutes). **Sampling site(s):** 2 sites- ▪ Head of the bed within 1m of the patient's head at a height of 1.3m ▪ Toilet of isolation	**Test:** RT-PCR **Threshold(s):** Ct<38 considered positive; however increased threshold to <45 due to low viral loads. **Gene Target(s):** ORF-1 or N	**SARS-CoV-2 RNA detection:** ▪ Detected in one air sample from ICU (near patient bed). ▪ Detected in three air samples from isolation ward (two from bathroom, one from ward) all of which were detected near the same patient.

		ward, less than 1m from toilet. **Number of samples:** 400 samples in total but unclear what proportion were air. **Environmental Conditions:** ICU unit- laminar flow originating in the ceiling and extracted through wall vents at bed level (average air changes per hour 240-360). Temperature, relative humidity reported and consistent. **Timing of sample collection:** Range of duration of illness to sample collection 43-57 days.		
Liu 2020 **China** **Air sampling study** **Published:** https://doi.org/10.1038/s41586-020-2271-3(2020)	**Setting:** General wards within one hospital and intensive care unit from a second hospital. Medical staff areas and public areas from both hospitals. **Patient demographics:** NR **Clinical characteristics:** Hospital one - patients with mild symptoms. Hospital two - patients with severe symptoms.	**Collection:** Presterilised gelatin filters (Sartorius, Germany) fixed flow rate of 5l per minute: ▪ Aerosol samples of total suspended particles (TSP) with no upper size limit to quantify RNA concentrations of SARS-CoV-2 in aerosol. **Sampling site(s):** 3 sites- ▪ Patient Areas , where the COVID-19 patients have physical presence. ▪ Medical Staff Areas the	**Test:** droplet digital PCR **Threshold(s):** Mean Ct was 21.23 in air samples **Gene Target(s):** ORF and N	**RNA concentration levels:** ▪ Patient areas: range 0-19 copies m^{-3} ○ Very low in isolation wards and ventilated patient rooms but it was elevated in patients' toilet areas (0-9 without toilet sample of 19). ▪ Medical staff areas: range 0-21 copies m^{-3} ○ Initially high concentrations of viral RNA but these levels reduced to undetectable levels after implementation of rigorous sanitisation procedures. ▪ Public areas: range 0-11 copies m^{-3} ○ Levels of airborne SARS-CoV-2 RNA in the majority of public areas was undetectable except in two areas

		workplaces in the 2 hospitals exclusively accessed by the medical staff who had direct contact with the patient. ▪ Public Areas venues open for the general public. All sampling instruments were located in the centre of the respective sampling area, where the sampling inlet was at a height of ~1.5 m from floor. **Number of samples:** 30 **Environmental conditions:** Negatively pressurised isolation and high air exchange rate inside ICU, CCU and ward rooms.		prone to crowding, possibly due to infected carriers in the crowd **Aerosol size:** ▪ SARS-CoV-2 aerosols were mainly found to include two size ranges, one in the submicrometre region ($0.25 \leq dp \leq 1.0$ μm) and the other in supermicrometre region ($dp > 2.5$ μm)
Ma 2020 **China** **Air sampling study** **Preprint:** https://doi.org/10.1101/2020.05.31.20115154	**Setting:** 39 patients (35 SARS-CoV-2 confirmed) from two hospitals and hotel quarantine facilities. **Patient demographics:** Sixty-one percent of the COVID-19 patients were aged under 40. **Clinical characteristics:** Forty percent had mild symptoms.	**Collection:** ▪ Exhaled breath condensate: BioScreen device ○ patients were instructed to exhale for 5 min towards the cooled hydrophobic film through a long straw ▪ Air sample: Two impingers depending on size of space. **Sampling sites:** Corridor, Hotel room, Hospital CT room, ICU room, Toilet	**Test:** RT- PCR **Threshold(s):** Ct<37 **Gene Target(s):** ORF and N	**SARS-CoV-2 RNA detection:** ▪ Exhaled breath condensate: ▪ Positive rate for samples was 16.7% (5/30). ▪ Estimated rate 10^3-10^5 RNA copies/min. ▪ Air samples: ▪ 3.8% (1/26) of air samples were positive for SARS-CoV-2. ▪ One sample from an unventilated quarantine hotel toilet room was positive.

		room, Emergency room, Clinical observation room, and Hospital ward. **Number of samples:** ▪ 26 air samples ▪ 30 exhaled breath condensate **Timing of sample collection:** Times from symptom onset to the EBC collection were all less than 14 days		
Ong 2020 **Singapore** **Air sampling study** **Published:** 10.1001/jama.2020.3227	**Setting:** Three patient spaces at a dedicated outbreak centre **Patient demographics:** NR **Clinical characteristics:** Two patients had moderate symptoms, one patient had mild symptoms	**Collection:** ▪ SKC Universal Pumps (4 hours at 5 L/min) in the room and anteroom ▪ Sartorius MD8 microbiological sampler (15 minutes at $6m^3/h$) outside the room **Sampling site(s):** Sites inside and outside patient rooms Samples taken from 2 patients with moderate symptoms after cleaning. Samples from patient with mild symptoms taken before cleaning. **Number of samples:** NR **Timing of sample collection:** Range from 4 to 11 days since symptom onset	**Test:** RT-PCR **Threshold(s):** Ct< 32 indicative of strong positive results. Ct>32 indicative of weak positive results. **Gene Target(s):** RdRp and E	**SARS-CoV-2 RNA detection:** No positive detection from air sampling.

		Environmental conditions: Air exchange likely diluted samples		
Razzini 2020 **Italy** **Air sampling study** **Published:** https://doi.org/10.1016/j.scitotenv.2020.140540	**Setting:** Isolation ward of a single hospital. **Patient demographics:** NR **Clinical characteristics:** Two patients were ventilated.	**Collection:** MD8 Airport Portable Air Sampler with Gelatine Membrane Filters (Sartorius, Varedo, MB, Italy) 40min with a flow of 50 l/min positioned 1.5m above the floor. **Sampling site(s):** 3 zones of the ward including contaminated (COVID-19 patients' area), semi-contaminated (undressing room), and clean areas. **Number of samples:** 37 **Environmental conditions:** Air conditioning system consisted of a negative airflow system. Temperature and relative humidity ranged from 20° to 22 °C and 40 to 60% respectively. **Timing of sample collection:** NR	**Test:** RT-PCR **Threshold(s):** Ct value ≤40 considered positive **Gene Target(s):** NR	**SARS-CoV-2 detection:** ▪ 20 air samples collected from ICU and corridor for patients were positive for viral RNA. ▪ No detection in the undressing room, dressing room, and passage/lockers area for staff.
Santarpia 2020 **United States** **Air sampling study** **Preprint:** https://doi.org/10.1101/2020.03.23.20039446	**Setting:** Ward unit and quarantine unit at single medical centre **Patient demographics:** 13 confirmed COVID-19 patients **Clinical characteristics:** 57.9% of patients recorded temperature, 57.9% reported other symptoms	**Collection:** Sartorius Airport MD8 air sampler at 50lpm for 15 minutes **Sampling sites:** Inside and outside patient rooms in varying locations but at least one metre away from patient	**Test:** RT-PCR **Threshold(s):** Ct<39.2 **Gene Target(s):** E **Cell culture:** Vero E6 cells were used to culture virus from samples	**SARS-CoV-2 RNA detection:** 63.2% of in-room air samples were positive (mean concentration 2.42 copies/L of air) 58.3% samples taken outside the rooms in the hallways were positive with a mean concentration of 2.51 copies/L of air. In one case, sampler was placed near the patient and one was placed >2 metres from the

	independent of temperature (primarily cough)	**Number of samples:** 32 **Timing of sample collection:** Ward unit day 10 of occupancy. Quarantine unit days 5-9 of occupancy		patient's bed while the patient was receiving oxygen (1L) via nasal cannula. Both samples were positive with the one closest to the patient indicating a higher airborne concentration of RNA (4.07 as compared to 2.48 copies/L of air). **Cell culture:** Due to the low concentrations recovered in these samples cultivation of virus was not confirmed in these experiments. In one air sample, cell culture indicated some evidence for the presence of replication competent virus
Santarpia 2020b **United States** **Air sampling study** **Preprint:** https://doi.org/10.1101/2020.07.13.20041632	**Setting:** Two mixed acuity wards. **Patient demographics:** NR **Clinical characteristics:** NR	**Collection:** NIOSH BC251 air sampler and Aerodynamic Particle Sizer Spectrometer (APS 3321; TSI, Inc., Shoreview, MN) was used to measure aerosol concentrations and size distributions (30 minutes continuous). **Sampling sites:** Collected in area around 6 COVID-19 patients. Air sampler placed at the foot of each patient's bed. **Number of samples:** 18 (six of each size: >4.1 µm, 1-4 µm, and <1 µm) **Environmental conditions:** NR **Timing of sample collection:** Range of time from admission to sampling 2-24 days. Range of time since COVID-19 confirmation and sampling 2-24 days.	**Test:** rRT-PCR **Threshold(s):** 20 $TCID_{50}$/mL of extracted sample **Gene Target(s):** E **Cell culture:** Cultivated in Vero-E6 cells (Dulbeccos's minimal essential medium). Definitive replication was considered to occur for rRT-PCR samples in which a significant increase in RNA was detected in the supernatant. Supplementary western blot and transmission electron microscopy analysis performed.	**SARS-CoV-2 RNA detection:** ▪ Detection of SARS-CoV-2 viral RNA in all three aerosol sizes from all six rooms (18/18). **Cell culture:** ▪ Statistically significant viral growth in 3 of the 18 samples (3/6 of <1 µm samples) after 5-6 days of incubation. ▪ 2/6 1-4 µm samples demonstrated viral growth, but did not reach statistical significance. ▪ The presence of SARS-CoV-2 was observed via western blot in four out of five samples.

Wong 2020 **Singapore** **Air sampling study** **Preprint:** https://doi.org/10.1101/2020.05.31.20107862	**Setting:** Single patient room of a hospital **Patient demographics:** Confirmed SARS-CoV-2 infection. Occupied room for one day **Clinical characteristics:** Assumed that the cases were symptomatic while residing in sites	**Collection:** VTM using a cyclonic air sampler with at 300L/min for 30 minutes **Sample site(s):** Two sites- Enclosed air-conditioned room that had no mechanical ventilation. Directly outside patient room. **Sample numbers:** Within room- 4, outside room- 2 **Timing of sample collection**: Premises were vacated at least 24 hours prior to sampling **Environmental Conditions:** Wall-mounted fan coil unit	**Test:** RT-PCR **Threshold(s):** NR **Gene Target(s):** RdRp	**SARS-CoV-2 RNA detection:** No positive detection from air samples
Yamagishi 2020 **Japan** **Air sampling study** **Preprint:** https://doi.org/10.1101/2020.05.02.20088567	**Setting:** Cabins of confirmed COVID-19 cases and non-cases on a cruise ship **Patient demographics:** NR **Clinical characteristics:** Symptomatic and asymptomatic cases	**Collection:** Airport MD8 (Sartorius) 50L/min for 20 minutes **Sampling site(s):** Various locations in case and non-case cabins. **Number of samples:** 14 **Timing of sample collection:** 1-17 days after cases had left cabins **Environmental Conditions:** Temperature and humidity values provided	**Test:** rRT-PCR **Threshold(s):** NR **Gene Target(s):** NR **Cell culture:** Inoculated on confluent VeroE6/TMPRSS2 cells	**SARS-CoV-2 RNA detection:** No positive SARS-CoV-2 RNA detection from air sampling
Zhou 2020 **China** **Air sampling study** **Preprint:** https://doi.org/10.1101/2020.05	**Setting:** Four hospitals **Patient demographics:** 10 recovering confirmed SARS-CoV-2 infected patients and three negative cases with influenza. Aged from 29 to 81, with 70% of	**Collection:** ▪ Exhaled breath condensate: BioScreen II device ▪ Air samples: Impinger	**Test:** rRT-PCR **Threshold(s):** Ct< 37 **Gene Target(s):** ORF and N	**SARS-CoV-2 RNA detection:** ▪ Air samples: ▪ No air samples tested positive for SARS-CoV-2

.31.20115196	the patients older than 50. **Clinical characteristics:** Six of the patients had experienced severe symptoms during the disease course	samplers **Sampling sites:** Various locations within wards **Number of samples:** ▪ Air samples: 44 ▪ Exhaled breath condensate: 9 **Environmental conditions:** Natural ventilation (1.6-3.3 m/s) **Timing of sample collection:** EBC collected at least 14 days since symptom onset		○ Likely due to the dilution or inactivation through applied disinfection. ▪ Exhaled breath condensate: ▪ 2/9 patients detectable SARS-CoV-2 in EBC (~10^5 RNA copies/m^3).
Zhou 2020b **United Kingdom** **Air sampling study** **Preprint:** https://doi.org/10.1101/2020.05.24.20110346	**Setting:** Teaching hospital group in London, five hospitals across four sites. Most sampling from one site with COVID-19 patients managed in cohort wards **Patient demographics:** NR **Clinical characteristics:** NR	**Collection:** Coriolis µ air sampler at 100-300 litres/minute **Sampling sites:** Seven clinical areas occupied by COVID-19 patients and one public area **Number of samples:** 31 **Environmental conditions:** NR **Timing of sample collection:** NR	**Test:** qRT-PCR **Threshold(s):** Where both of the PCRs performed from an air or surface sample detected SARS-CoV-2 RNA as positive, and samples where 1 of the 2 PCRs performed from an air or surface sample detected SARS-CoV-2 RNA as suspected. **Gene Target(s):** E **Cell culture samples:** VeroE6 and Caco2	**SARS-CoV-2 RNA detection:** ▪ Detected in 14/31 (38.7%) air samples ▪ Detected in air samples from all eight areas tested with levels ranging from 10^1 to 10^3 genome copies / m^3 **Cell culture:** No virus was cultured from air samples.

Table 3. Summary of Microbiological studies

Author Country Study design Status: DOI	Experimental details	Analysis method	Results
Fears 2020 **United States** **Microbiological study** **Published (as early release):** 10.3201/eid2609.201806	**Strain:** 2019-nCoV/USA-WA1/2020 **Aerosol generation:** Collison 3-jet (C3), Collison 6-jet (C6), and Aerogen Solo. Automated Bioaerosol Exposure System. Aerosol size distributions produced by the generators used, in mass median aerodynamic diameter, were 1–3 µm. **Environmental condition(s):** 23°C ± SD 2°C and 53% ± SD 11% relative humidity throughout the aerosol stability experiment.	**Collection:** ▪ Custom-built rotating (Goldberg) drum with yimed aerosol samples from the drum at 10 min, 30 min, 2 hrs, 4 hrs, and 16 hrs after initiation. ▪ Quantified virus contents by plaque assay and reverse RT-qPCR. ▪ Quantified virion integrity with electron microscopy. **Analysis:** ▪ Calculated the dynamic aerosol efficiency or spray factor (Fs) as a unitless quotient of initial titer (PFU/L in liquid stock) to the resulting aerosol (PFU/L aerosol) providing a quantitative indicator for comparing airborne fitness. ▪ Graphed plaque assay and RT-qPCR results and applied nonlinear least-squares regression analysis single-order decay.	**SARS-CoV-2 detection:** SARS-CoV-2 detected at all time points during the aerosol suspension stability experiment. Minor but constant fraction of SARS-CoV-2 maintained replication-competence at all time points including when sampled after 16 hrs of aerosol suspension. **Virus decay:** Flat decay curve when measured for infectivity and failed to provide a biologic half-life (κ = 2.93E-06; t1/2 = 2.36E+05; τ = 3.40E+05). RT-qPCR showed minimal decreases in aerosol concentration measured in viral genome copies across all of time points sampled and approximated the decay curve of the infectious virus fraction, including similar decay curve characteristics. **Electron microscopy:** Virions aged for 10 min or 16 hrs were similar in shape and general appearance to virions examined in samples of viral inoculum collected before aerosolisation suggests the potential to be infectious after long-term aging in aerosol suspension. **Cell culture:** Not performed. Viability/infectivity was hypothesised by quantitative measurement of viral airborne efficiency augmented by assessment of virion morphology.
Schuit 2020 **United States** **Microbiological study**	**Strain:** SARS-CoV-2 (BetaCoV/USA/WA1/2020) **Aerosol generation:** 2 different	**Collection:** ▪ 10 second sample was collected using	**Decay rates:** ▪ Average decay constants for infectivity ranged from near zero for tests without simulated sunlight to 0.48 min-1, or 38%/min,

Published: 10.1093/infdis/jiaa334/5856149	environmentally controlled rotating drum aerosol chambers, with volumes of 16-L and 208-L. Five samples of the aerosol present in a drum were collected over the course of each test. **Environmental condition(s):** Relative humidity, and simulated sunlight. ▪ Relative humidity was controlled by adjusting the balance of dry and humid air entering the drum. Levels 20, 45, and 70%. ▪ Simulated sunlight generated by a solar simulator (Newport Oriel) equipped with a 320-nm highpass filter. Simulated sunlight intensities-darkness, mid-intensity, and high-intensity. **Cells:** Vero Cells were grown at 37 °C and 5% CO2 in culture medium.	an Aerodynamic Particle Sizer. ▪ 20 to 60 second sample was collected onto a 25 mm gelatin filter in a Delrin filter holder operated at 5 L/min. The gelatin filter was removed from the holder and dissolved in 10 mL of culture medium to re-suspend the collected virus. ▪ Titres of infectious virus in aerosol samples were determined by microtitration assay on confluent monolayers of Vero cells. **Analysis:** For each test, time-series log_{10} transformed viral and mass aerosol concentration data were fit using linear regression. The slopes of these regression lines represent the decay rates of infectious virus and total aerosol mass in the chamber, respectively.	for tests with high-intensity simulated sunlight at 70% relative humidity. ▪ Stepwise regression analysis demonstrated that $k_{Infectivity}$ was dependent on the simulated sunlight intensity and the suspension matrix ($p<0.0001$ and $p=0.0004$, respectively), but not relative humidity ($p=0.095$).
van Doremalen 2020 **United States** **Microbiological study** **Published:** 10.1056/NEJMc2004973	**Strain:** SARS-CoV-2 nCoV-WA1-2020 (MN985325.1) **Aerosol generation:** Aerosols (<5 µm) containing SARS-CoV-2 (105.25 50% tissue-culture infectious dose [TCID50] /ml) or SARS-CoV-1 (106.75-7.00 TCID50/ml) were generated with the use	**Analysis:** Estimated the decay rates of viable virus titres using a Bayesian regression model.	**Virus viability:** SARS-CoV-2 remained viable in aerosols throughout the duration of experiment (3 hours), with a reduction in infectious titre from $10^{3.5}$ to $10^{2.7}$ $TCID_{50}$ /L of air. Reduction was similar to that observed with SARS-CoV-1, from $10^{4.3}$ to $10^{3.5}$ $TCID_{50}$ /ml. **Half-life:** ▪ Median 1.1 for SARS-CoV-2 (95% credible intervals of 0.64 to

	of a three-jet Collison nebulizer and fed into a Goldberg drum to create an aerosolized environment. **Ct values:** Values between 20 and 22.		2.64). ▪ Median 1.2 for SARS-CoV-1 (95% credible intervals of 0.78 to 2.43).
Yu 2020 **United States** **Microbiological study** **Preprint:** https://doi.org/10.1101/2020.06.13.150243	**Strain:** SARS-CoV-2 USA-WA1/2020 **Aerosol generation:** 6 jet automated aerosol control platform. **Environmental condition(s):** Novel commercial nickel folded foam filter, heated to 250 °C.	Bioaerosol samples were collected before and after filtration for each aerosol run using S.K.C. BioSamplers. Vero cells were cultured in Dulbecco's Modified. Eagle Medium with 10% fetal bovine serum and 1% penicillin/streptomycin.	**Virus viability:** 2.7-fold log reduction $TCID_{50}$ was noted when the filter was heated to ~200 °C with estimated 99.8% viral load reduction from upstream to downstream in the device using a single pass-through when the filter was heated up to 200 °C.

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