



Review

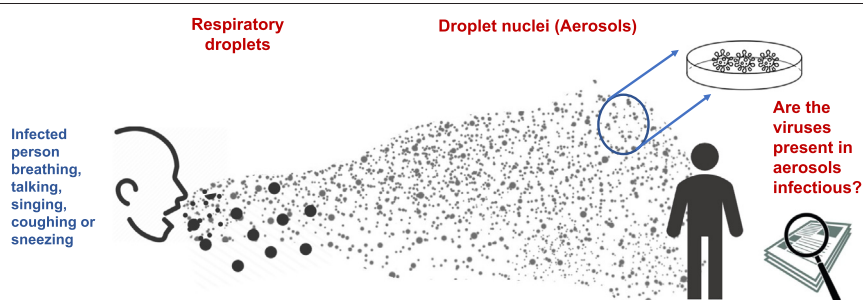
Airborne spread of infectious SARS-CoV-2: Moving forward using lessons from SARS-CoV and MERS-CoV

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HIGHLIGHTS

- Detection of SARS-CoV-2 RNA in the air do not correlate to infectivity.
- Virus viability in air is important to understand the aerosol transmission.
- SARS-CoV-2 may be less stable in higher temperatures and relative humidity.
- The effect of temperature and humidity on SARS-CoV-2 seems to be residual.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Although an increasing body of data reports the detection of SARS-CoV-2 RNA in air, this does not correlate to the presence of infectious viruses, thus not evaluating the risk for airborne COVID-19. Hence there is a marked knowledge gap that requires urgent attention. Therefore, in this systematic review, viability/stability of airborne SARS-CoV-2, SARS-CoV and MERS-CoV viruses is discussed.

Methods: A systematic literature review was performed on PubMed/MEDLINE, Web of Science and Scopus to assess the stability and viability of SARS-CoV, MERS-CoV and SARS-CoV-2 on air samples.

Results and discussion: The initial search identified 27 articles. Following screening of titles and abstracts and removing duplicates, 11 articles were considered relevant. Temperatures ranging from 20 °C to 25 °C and relative humidity ranging from 40% to 50% were reported to have a protective effect on viral viability for airborne SARS-CoV and MERS-CoV. As no data is yet available on the conditions influencing viability for airborne SARS-CoV-2, and given the genetic similarity to SARS-CoV and MERS-CoV, one could extrapolate that the same conditions would apply. Nonetheless, the effect of these conditions seems to be residual considering the increasing number of cases in the south of USA, Brazil and India, where high temperatures and humidities have been observed.

Conclusion: Higher temperatures and high relative humidity can have a modest effect on SARS-CoV-2 viability in the environment, as reported in previous studies to this date. However, these studies are experimental, and do not support the fact that the virus has efficiently spread in the tropical regions of the globe, with other transmission routes such as the contact and droplet ones probably being responsible for the majority of cases reported in these regions, along with other factors such as human mobility patterns and contact rates. Further studies are needed to investigate the extent of aerosol transmission of SARS-CoV-2 as this would have important implications for public health and infection-control policies.

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1. Introduction

Coronaviruses are enveloped positive-strand RNA viruses from the *Coronaviridae* family. Seven members have been reported to infect humans, including HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, Severe Acute Respiratory Syndrome Virus (SARS-CoV), Middle East Respiratory Syndrome Virus (MERS-CoV) and the emerging SARS-CoV-2 (responsible for COVID-19) (Shereen et al., 2020; Ye et al., 2020). Coronaviruses usually infect the cells from the respiratory tract and are responsible for different respiratory diseases that range from mild disease to severe acute respiratory syndromes (Rothan and Byrareddy, 2020; Talbot et al., 2008). Human coronaviruses represent a major problem for human health and impose a tremendous economic burden (Keogh-Brown and Smith, 2008; Paules et al., 2020). These viruses are considered a leading cause of morbidity and mortality in humans worldwide, as seen with the past SARS and MERS outbreaks (Kim et al., 2017; Qiu et al., 2018) and the current COVID-19 global pandemic (Peeri et al., 2020). Globally, as of 10:52 am CEST, 24 September 2020, there have been 31,664,104 confirmed cases of COVID-19, including 972,221 deaths, reported to the World Health Organization (WHO) (WHO, 2020a).

Viral respiratory infections are known to be spread by contact (direct or indirect) with secretions expelled by the infected person, or through air via droplets and aerosols (Kutter et al., 2018). Contact transmission can happen when a healthy person comes in close contact with an infected person (direct contact) or surfaces (fomites) where virus-containing droplets expelled by an infected person have been deposited (indirect contact) (Morawska and Cao, 2020). Transmission of viruses through air can happen via droplets or aerosols generated during coughing, sneezing, talking, singing or breathing (Jones and Brosseau, 2015), as well as during aerosol-generating medical procedures (WHO, 2020b).

Respiratory droplet transmission can occur when a person is in close contact (within 1 m) with an infected person who is coughing, talking, sneezing or singing; in these circumstances, respiratory droplets that contain the virus can reach the mouth, nose or eyes of a susceptible person and might result in infection. Airborne transmission is defined as the spread of an infectious agent caused by the dissemination of aerosols that remain infectious when suspended in air over long distances and time (WHO, 2020c). These small particles of pathogen-containing respiratory secretions expelled into the air can remain airborne for long periods (Atkinson et al., 2009), carrying their contents away from where they were originated (Siegel et al., 2007), therefore raising the possibility of airborne transmission also in the absence of aerosol-generating procedures.

According to the most recent interim guide by WHO (June 2020), with advice on the use of masks in the context of COVID-19 (WHO, 2020b), there is available evidence for SARS-CoV-2 transmission by close contact through respiratory droplets, whereas airborne transmission is limited to aerosol-generating procedures in the clinical care of patients with COVID-19 (Brurberg, 2020; Wilson et al., 2020;

Workman et al., 2020). On July 6th 2020, a commentary, supported by a group of 239 scientists from all over the world was published, raising alarm for airborne transmission and urged the medical community and public health authorities to acknowledge the potential for airborne transmission and add preventive measures to avoid transmission through this route (Morawska and Milton, 2020). Following that, on July 9th 2020, the WHO issued a scientific brief on viral transmission (WHO, 2020c) and stated that more research is needed given the possible implications of an airborne route of transmission, although saying that short-range aerosol transmission cannot be ruled out in crowded, poorly ventilated spaces.

Although there is significant evidence for aerosol transmission, the scientific community is still trying to answer the question of whether SARS-CoV-2 might also spread through aerosols in the absence of medical aerosol-generating procedures, with the airborne spread of infectious SARS-CoV-2 hypothesis still not fully understood (WHO, 2020b; Morawska and Cao, 2020).

One of the biggest obstacles in fully clarifying the airborne transmission of SARS-CoV-2 is that most studies performed only focused on the detection of viral RNA and do not correlate to the infectivity of these viral particles. There is an inherent high technical complexity that also hampers the confirmation of the aerosolized SARS-CoV-2 infectiousness, requiring viral replication to differentiate viable from non/viable virus and including a number of particular methodological requirements, namely proper specimen selection, collection, transport, and storage that preserve viral infectivity (Leland and Ginocchio, 2007).

Moreover, both the WHO and the Centers for Diseases Control and Prevention (CDC) provided recent guidelines recommending that handling of material with high concentrations of viable SARS-CoV-2, such as when performing virus propagation, should be performed only in laboratories capable of meeting strict containment requirements and practices (Bio-safety Level-3), limiting the number of institutions capable of assessing aerosolized SARS-CoV-2 viability (Blacksell et al., 2020; CDC, 2020).

Considering the many structural and genetic similarities between SARS-CoV, MERS-CoV, and SARS-CoV-2 (Petrossillo et al., 2020), and taking into consideration previous studies about SARS-CoV and MERS-CoV that point out the potential for airborne transmission of these viruses (Eissenberg et al., 2020; Kutter et al., 2018; Olsen et al., 2003; Pyankov et al., 2018; Qian and Zheng, 2018; Ramanathan et al., 2020; Tellier et al., 2019; Yu et al., 2004; Zhao et al., 2011), the likelihood for airborne transmission of SARS-CoV-2 is very high (Morawska and Cao, 2020; Tellier et al., 2019). However, to date, only five published studies have provided information on SARS-CoV-2 viability in air (Binder et al., 2020; Lednický et al., 2020a, 2020b; Santarpia et al., 2020b; van Doremalen et al., 2020). Thus, there is a marked knowledge gap that requires urgent attention. An opportunity for advancing research in airborne transmission of SARS-CoV-2 is by comparison to the viability of SARS-CoV and MERS-CoV. Therefore, in this systematic review, the viability/stability of aerosols containing SARS-CoV and MERS-CoV viruses will be discussed to provide information on potential mitigation strategies for SARS-CoV-2 airborne transmission.

2. Materials and methods

The present review includes studies published in the past 18 years (1 January 2002 to 25 September 2020), since the emergence of SARS-CoV (WHO, 2002) and MERS-CoV (WHO, 2012), in the following databases: PubMed/MEDLINE, Web of Science and Scopus. No language restrictions were imposed during the search, retrieving only one article in Chinese. With no prior review articles on this topic, an exhaustive search was made, and published research articles were included.

The following search terms were used: "SARS", "MERS", "airborne", "viability", "stability", "virus", "aerosol", "coronavirus", and "air sample". A total of 27 articles were found with potential interest from the initial search and their titles were screened based on their context of research. From those, 20 articles remained, and their abstracts were appropriately reviewed. After this, exclusions were performed based on the following criteria: i) if the virus studied was SARS-CoV, MERS-CoV or SARS-CoV-2; and ii) if the viability of the virus sampled from air was assessed. Using these criteria, 18 articles were excluded and 10 additional relevant articles were found while reading the selected articles, with 1 article being excluded. Summarizing, 11 articles were reviewed in detail. Fig. 1 shows the flowchart with the number of studies identified and included/excluded following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009).

The databases were independently screened by all authors, and relevant information was extracted. Differences on opinions about whether to include an article or not were solved by consensus between all the authors.

3. Results and discussion

The selected articles evaluated concerning the objective of the research, sampling site/methods and main conclusions are compiled in Table 1.

3.1. Viral infectivity is difficult to be assessed in the present SARS-CoV-2 pandemic

Viral infectivity is defined as the capacity of the virus to attach and enter the host cell and use its resources to ultimately produce new infectious virions (Rodríguez et al., 2009). In the case of enveloped viruses such as coronaviruses, viral entry is initiated by the interaction of the viral particle with specific proteins on the cell surface. After initial binding of the receptor, these enveloped viruses fuse their envelope with the host cell membrane to deliver their capsid to the target cell (Belouzard et al., 2012). The capsid also confers protection to the viral genome by preventing its degradation by nucleases and other abiotic stresses. Therefore capsid integrity is a critical attribute for the virus to successfully infect a host cell (Cliver, 2009).

Among the reviewed literature, only a few papers explored viral viability in air samples (Agranovski et al., 2004; Binder et al., 2020; Booth et al., 2005; Kim et al., 2016; Lednický et al., 2020a, 2020b; Pyankov et al., 2018; Santarpia et al., 2020b; van Doremalen et al., 2013, 2020; Xiao et al., 2004). Remarkably, the majority of the literature focuses exclusively on the detection of viral RNA in air samples (Cheng et al., 2020; Chia et al., 2020; Faridi et al., 2020; Guo et al., 2020; Ong et al., 2020), which does not necessarily mean that these aerosols contain viable/infectious viruses that could be transmitted and infect other people. Thus, there is a significant knowledge gap concerning the significance of the results for public health (Leifels et al., 2015).

Aiming at simplifying the determination of viral infectivity, alternative strategies to cell culture and TCID₅₀ determination have been explored. These strategies resort typically combining RT-PCR with a pre-processing step aiming at deconvoluting viable from non-viable virus particles prior to amplification (Goyal and Cannon, 2006). A few examples of these methods are (1) enzymatic

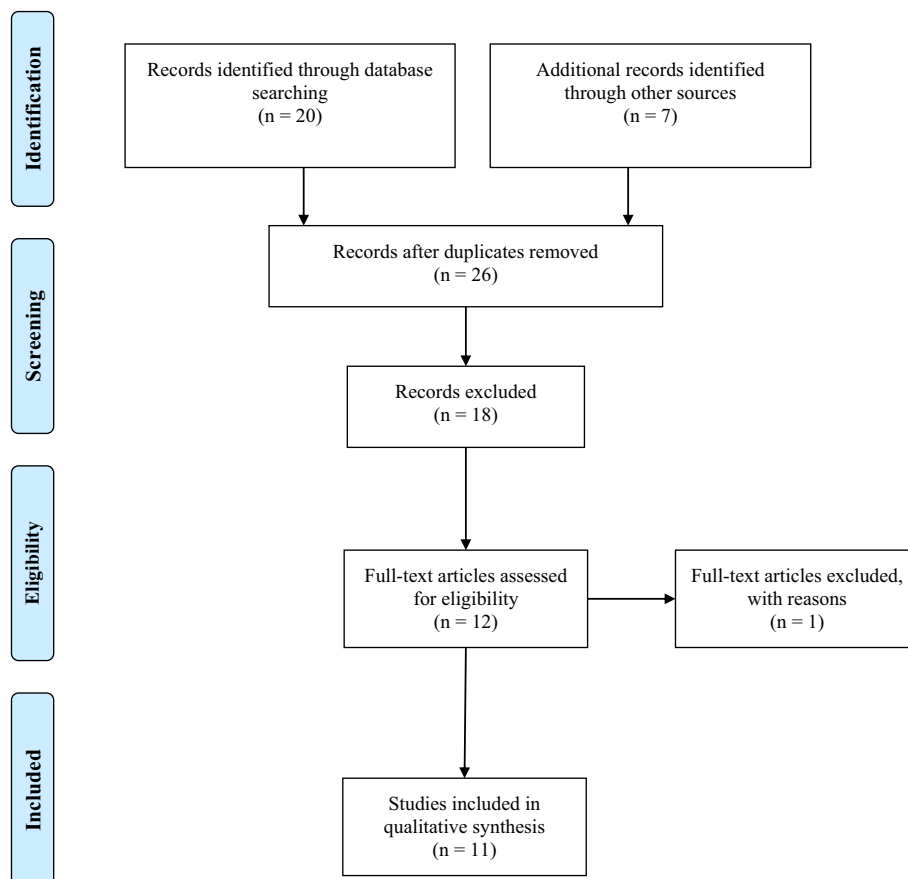


Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart.

Table 1
Articles reporting details on viability of SARS-CoV-1, MERS-CoV and SARS-CoV-2 in air.

	Reference	Targeted virus	Objective	Sampling		Main outcomes			
				Site	Method	Virus presence/quantification	Viability	Conditions for viability	
Real-world sampling studies	Agranovski et al. (2004)	SARS-CoV-1	To explore the feasibility of a new personal bioaerosol sampler for monitoring of viable airborne SARS virus.	PC4 facility with HEPA filters installed in the pipeline connecting sampler and vacuum pump to prevent the equipment contamination.	Contaminated air was bubbled through porous medium submerged into liquid and subsequently split into multitude of very small bubbles. The particles are scavenged by these bubbles, and, thus, effectively removed.	Natural decay of the virus in the collection fluid was around 0.75 and 1.76 log during 2 and 4 h of continuous operation, respectively. A much higher decay rate (2.58 log) was observed for the bubbling through viral suspension in sterile water.	Yes.	The device filled with virus maintenance fluid was capable of providing a relatively low level of microbial decay and can be evaluated for monitoring of such microorganisms in the air.	
	Xiao et al. (2004)	SARS-CoV-1	To assess the risk of aerosol transmission in SARS patients admitted to a hospital through testing the air samples.	Air samples were collected from 7 wards and 1 balcony of the hospital, 3 times a day for 3 continuous days.	The bioaerosol sampler type FA-2 was used. RT-PCR was used to amplify the N protein gene of the SARS-CoV. The residual solutions were inoculated into prepared cell cultures to isolate live virus. The positive samples were then identified by indirect immunofluorescence assay and sequence analysis of the PCR products.	Positive rates of RT-PCR of air samples were 29.03% in the wards and 20.0% in balcony respectively. Viable isolate was obtained from one of the 36 samples. The isolate could cause typical cytopathic effects similar to those SARS-CoV on Vero-E6 cells and the effects could be stably passed. Indirect immunofluorescence assay showed positive with the serum of a SARS patient.	Yes.	Not specified.	
	Booth et al. (2005)	SARS-CoV-1	To investigate environmental contamination in SARS units during the Toronto outbreaks of SARS by employing novel air sampling and conventional surface swabbing.	Environmental samples were collected from 19 rooms in the SARS units of 4 healthcare facilities where patients with SARS were staying.	Air sampling was performed using a high-resolution slit-sampler system and samples were tested for the presence of SARS-CoV by RT-PCR and cell culture isolation.	The samples were tested by viral culture with reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence assay (IFA) using MERS-CoV Spike antibody, and electron microscopy (EM).	PCR-positive viruses were collected from wet and dry air samples but results of viability assays of the samples for infectivity in Vero-E6 cell culture were negative.	No.	Not specified.
	Kim et al. (2016)	MERS-CoV	To study the possible contribution of contaminated hospital air and surfaces to MERS transmission.	Two hospitals treating MERS-CoV patients in Seoul.	The samples were tested by viral culture with reverse transcription polymerase chain reaction (RT-PCR) and immunofluorescence assay (IFA) using MERS-CoV Spike antibody, and electron microscopy (EM).	The presence of MERS-CoV was confirmed by RT-PCR of viral cultures of 4 of 7 air samples. In addition, MERS-CoV was detected in 15 out of 68 surface swabs by viral cultures. IFA on the cultures of the air and swab samples revealed the presence of MERS-CoV. EM images also revealed intact particles of MERS-CoV in viral cultures of the air and swab samples.	Yes.	Not specified.	
Lednický et al. (2020a)	SARS-CoV-2	To assess whether SARS-CoV-2 can remain viable in aerosols.	A hospital room from a designated COVID-19 ward.	Air samples were collected using a prototype VIVAS air sampler, as well as a BioSpot-VIVAS BSS300P that collects airborne particles via a water-vapour condensation method. The virus was further inoculated in LLC-MK2 and Vero E6 cells to assess viability.	SARS-CoV-2 genomic RNA was detected by RT-PCR in material collected by air samplings. Virus-induced CPE was observed in LLC-MK2 and Vero E6 cells inoculated with material extruded from the specimen of one patient, and from liquid collection media from air samples. Furthermore, RT-PCR	Yes.	Not specified.		

Table 1 (continued)

Reference	Targeted virus	Objective	Sampling		Main outcomes			
			Site	Method	Virus presence/quantification	Viability	Conditions for viability	
Lednický et al. (2020b)	SARS-CoV-2	To detect SARS-CoV-2 RNA in air samples and check its viability, as well as analyzing the viral genomic sequence.	A clinic within a university student health care center.	Air samples were collected using the VIVAS air sampler. Virus was further inoculated in Vero E6 cells to assess viability.	analysis indicated that the LLC-MK2 and Vero E6 cultures inoculated with collection media from air samples contained SARS-CoV-2. RT-PCR analysis detected viral RNA in one air sample and the amount of virus present in 390 L of sampled air was low. Virus-induced CPE was observed within two days post-inoculation of Vero E6 cells with collection media from two air samples. However, RT-PCR for SARS-CoV-2 RNA from cell culture were negative.	Not sure ^a .	Not specified.	
Binder et al. (2020)	SARS-CoV-2	To study hospitalised COVID-19 patients, their hospital rooms (fomites and aerosols), and their close contacts for molecular and culture evidence of SARS-CoV-2 virus.	An empty hospital room (no patient contact for four days) in the Duke University Hospital COVID-19 ward.	Air samples were collected using NIOSH BC 251 aerosol samplers. Virus was further inoculated in Vero E6 cells to assess viability.	The prevalence of SARS-CoV-2 RNA in fomites and aerosols was low. Furthermore, no infectious virus was cultured from aerosol samples.	No.	Not specified.	
Santarpia et al. (2020b)	SARS-CoV-2	To study the aerosol and surface contamination with SARS-CoV-2 as well as viability/infectivity of the sampled virus.	Quarantine and isolation care rooms of the University of Nebraska Medical Center.	Air samples were collected using a Sartorius Airport MD8 air sampler. Virus was further inoculated in Vero E6 cells to assess viability.	It was found that 63.2% of in-room air samples were positive by RT-PCR to SARS-CoV-2. Furthermore, in two of the samples, cell culture indicated some evidence for the presence of replication competent virus.	Not sure ^a .	Not specified	
Laboratory studies	van Doremalen et al. (2013)	MERS-CoV	To study the stability of MERS-CoV under different environmental conditions, namely: at 20 °C – 40% RH; 30 °C – 30% RH and 30 °C – 80% RH.	Laboratory under controlled conditions.	To study environmental virus stability: 100 µl of 10 ⁶ TCID ₅₀ of MERS-CoV virus was spotted in droplets of 5 µl on the surface of steel or plastic washers and incubated at the desired conditions in an environmental chamber. To study aerosol stability: MERS-CoV was aerosolized aerosolised at 20 °C with 40% or 70% RH. Aerosol experiments were performed using the Aero MP aerosol management platform. Aerosols were collected continuously during aerosolisation in tissue culture media with an All Glass Impinger. Collected aerosols were analyzed for the presence of virus by RT-PCR and by virus end-point titration.	MERS-CoV was more stable at low temperature/low humidity conditions and could still be recovered after 48 h. During aerosolisation of MERS-CoV, no decrease in stability was observed at 20 °C – 40% RH.	Yes.	Low temperature and RH conditions.
	Pyankov et al. (2018)	MERS-CoV	To investigate the inactivation of airborne pathogenic MERS-CoV	Laboratory under controlled conditions.	A suspension containing virus was prepared and	At the lower temperature, the virus demonstrated high	Yes.	Low temperature and medium RH.

(continued on next page)

Table 1 (continued)

Reference	Targeted virus	Objective	Sampling		Main outcomes		
			Site	Method	Virus presence/quantification	Viability	Conditions for viability
		virus under controlled laboratory conditions, namely: representing common office environment (25 °C and 79% RH) and (2) climatic conditions of the Middle Eastern region where the virus was originated from (38 °C and 24% RH).		aerosolized aerosolised to the experimental aerosol chamber by a 3-jet Collision nebulizer nebulizer at the flow rate of 6 l/min of HEPA-filtered compressed air over 2 min time. Then the nebulizer nebulizer was switched off. The experiments were performed for two sets of parameters of the air. On completion of sampling at each time interval, the bioaerosol samplers were disconnected and aliquots of collecting liquid were acquired and analyzed analyzed by end-point titration in Vero E6 cells.	robustness and strong capability to survive with about 63.5% of viruses remaining infectious 60 min after aerosolisation. Virus decay was much stronger for hot and dry air scenario with only 4.7% survival over 60 min procedure.		
van Doremalen et al. (2020)	SARS-CoV-2	To evaluate the stability of SARS-CoV-2 and SARS-CoV-1 in aerosols and on various surfaces and estimate their decay rates using a Bayesian regression model.	Laboratory under controlled conditions.	Aerosols (<5 µm) containing SARS-CoV-2 (10 ^{5.25} 50% TCID ₅₀ per ml) or SARS-CoV-1 (10 ^{6.75-7.00} TCID ₅₀ per ml) were generated with the use of a three-jet Collision nebulizer nebulizer and fed into a Goldberg drum to create an aerosolized aerosolised environment. All samples were quantified by end-point titration on Vero E6 cells. SARS-CoV-2 nCoV-WA1-2020 (MN985325.1) and SARS-CoV-1 Tor2 (AY274119.3) were the strains used.	SARS-CoV-2 remained viable in aerosols throughout the duration of the experiment (3 h), with a reduction in infectious titer from 10 ^{3.5} to 10 ^{2.7} TCID ₅₀ per liter of air.	Yes.	Not specified.

*Abbreviations used in the table: HEPA - High Efficiency Particulate Arrestance, SARS - Severe Acute Respiratory Syndrome, RT-PCR - Reverse Transcription Polymerase Chain Reaction, qRT-PCR - Quantitative Reverse Transcription Polymerase Chain Reaction, TCID₅₀ - Media Tissue Culture Infective Dose, RH - Relative Humidity, MERS - Middle-East Respiratory Syndrome, IFA - Immunofluorescence Assay, EM - Electron Microscopy.

^a These experiments could assess CPE in cell culture, which is indicative of replication-competent virus, however, they were not able to obtain positive RT-PCR results from the supernatant of the cell culture.

pre-treatments (such as ribonuclease) (Escudero-Abarca et al., 2014; Monteiro and Santos, 2018; Nuanualsuwan and Cliver, 2002; Rönnqvist et al., 2014); (2) pre-treatments with intercalating dyes for detection of damaged capsids (Leifels et al., 2015; Moreno et al., 2015; Parshionikar et al., 2010; Randazzo et al., 2018); (3) porcine gastric mucin binding (Dancho et al., 2012; Kingsley et al., 2014); (4) antibody binding (Ogorzaly et al., 2013); and (5) integrated cell-culture PCR assays (Blackmer et al., 2000; Dunams et al., 2012). Overall, developments in these alternative viral infectivity analytical strategies, largely unexplored in the context of current and past coronavirus pandemics, is of paramount importance to enable not only routine fundamental insights into the effective spread of the virus and its societal impact, as well as enabling effective bio-sensing strategies for on-site determination of viral infectivity. Both these currently unopened avenues are critical to uncover the true impact of airborne spread of SARS-CoV-2.

3.2. Standardization of aerosol generation and sampling methods is required

A few of the reviewed papers resorted to high-powered jet nebulizers for aerosol generation as in the case of the experiment setting of van Doremalen et al. (2020), therefore not reflecting the real human-generated aerosol conditions where usually much larger particles are generated during a cough (Atkinson et al., 2009). This experiment can be instead interpreted as theoretical evidence that SARS-CoV-2 might be able to survive as droplet nuclei after an aerosol-generating medical procedure. van Doremalen et al. (2020) was the first group investigating the viability of aerosolized SARS-CoV-2, showing that the virus remained viable in aerosols for 3 h. Since then, four other studies attempted to assess SARS-CoV-2 viability in air samples through culture in Vero E6 and LLC-MK2 cells (Lednický et al., 2020a), and Vero E6 cells (Binder et al., 2020; Lednický et al., 2020b; Santarpia et al., 2020b), with

three of them reporting virus viability as the presence of CPE in the cell cultures. However, only one of these studies was able to show positive RT-PCR results from the supernatant of the cell cultures (Lednicky et al., 2020a). Moreover, several other studies that took place in clinical settings reported high positive rates of viral RNA in fomites and aerosol samples, with evidence of infectious virus being implied in aerosol samples (Chia et al., 2020; Razzini et al., 2020; Santarpia et al., 2020a; Zhou et al., 2020).

Previous work with SARS-CoV showed that viral RNA, as well as viable virus, were found in air samples (Booth et al., 2005; Xiao et al., 2004). Several other studies have reported that SARS-CoV airborne transmission was the main transmission route in indoor cases studied in Hong Kong's Prince of Wales Hospital (Li et al., 2005; Xiao et al., 2017; Yu et al., 2005), health care facilities in Canada (Booth et al., 2005) and in aircraft (Olsen et al., 2003). These results suggest that both SARS-CoV and SARS-CoV-2 can potentially be transmitted by aerosols and cause disease, therefore supporting potential airborne transmission.

The presence of MERS-CoV was also confirmed by RT-PCR of viral cultures of 4 out of 7 air samples from two hospitals in South Korea (Kim et al., 2016), and showed to be very stable in aerosol at 20 °C and 40% relative humidity (van Doremalen et al., 2013). Furthermore, the virus demonstrated relatively high robustness in the airborne form under controlled laboratory conditions (Pyankov et al., 2018), suggesting that MERS could also be transmitted by aerosols.

Although not investigating SARS-CoV-2 viability, some studies suggested that airborne transmission might occur (Buonanno et al., 2020; Cai et al., 2020; Hamner et al., 2020; Li et al., 2020a, 2020b). Li et al. (2020a) reported that up to 73% of infected patients reported having had no contact with a person with respiratory symptoms or exposure to relevant contaminated areas, which could be explained by a possible airborne transmission of the virus. Ong et al. (2020) also showed that air outlet fans located high on the wall behind the bed of one infected patient were contaminated with SARS-CoV-2, suggesting that virus-containing aerosols produced by the isolated patient were displaced by airflow and deposited on the vents.

Moreover, sampling methods and environmental conditions are very important factors to consider when studying viral viability and stability in aerosols because air sampling techniques can also affect the viability of virus recovered from air (Tseng and Li, 2005; Verreault et al., 2008). Problems such as inefficiency at the collection of fine particles, dehydration of viruses during the collection process, damage of the viruses during collection due to impaction forces resulting in the loss of viability of some or all the collected viruses, re-aerosolization leading to the loss of viruses from the collection media, and losses due to viruses being trapped by the inlet or the samplers' wall should be taken into consideration when interpreting the results of experiments involving air sampling. Noteworthy, samplers based on technologies such as the water-based condensation are considered more suitable for these studies (Pan et al., 2016; Yu et al., 2018).

3.3. Conditions that impact the infectivity of airborne viral particles

Physical characteristics of the environment such as ultraviolet light (UV), temperature, relative humidity, as well as wind currents and ventilation systems, are critical environmental factors that will determine the settling time of airborne particles (Alonso et al., 2015).

There are three types of UV light: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). UVC is known to be absorbed by RNA and DNA bases, resulting in the photochemical fusion of two adjacent pyrimidines into covalently linked dimers, which in turn lose the ability to pair with each other (Perdiz et al., 2000). Previous studies have shown that UVC is able to inactivate aerosolized coronaviruses (Darnell et al., 2004; Walker and Ko, 2007), with more recent studies on the subject reporting that simulated sunlight is also able to inactivate airborne SARS-CoV-2, highlighting the hypothesis that persistence and exposure

risk to airborne viruses might vary between indoor and outdoor environments (Ratnesar-Shumate et al., 2020; Schuit et al., 2020).

Temperature is another significant factor for virus survival because it can affect the state of viral proteins and the virus genome (Price et al., 2019). Temperatures above 60 °C for more than 60 min are thought to be sufficient to inactivate most enveloped viruses, and depending on the presence of any surrounding organic material such as saliva, the virus might be insulated against extreme environmental changes (Tang, 2009). In a study by Pan et al. (2019), it was reported that artificial saliva could better protect infectious viruses from deactivation by preventing viruses of reaching the air-water-interface, possibly due to the complex structure of the mucin component. In a similar study, Woo et al. (2012) reported that the inactivation efficiency of droplet and aerosolized viruses under different humidity levels and UV irradiation at a constant intensity were low in artificial saliva, indicating that solids present in it might exhibit a protective effect.

The relative humidity is also significant for virus survival and stability because phospholipid-protein complexes in enveloped viruses are usually more likely to denature in the air at medium to high relative humidity. In contrast, the protein coats of non-enveloped viruses denature easier at low relative humidity (Sobsey and Meschke, 2003), which explains why most enveloped viruses tend to survive longer at a lower relative humidity (Tang, 2009). In addition to that, when faced with high humidity, such as in tropical regions as the Amazon rainforest where relative humidity values can get close to 100% during the rainy season, viruses are associated with larger droplets that settle down much faster, which can be a limiting factor to transmission (Yang and Marr, 2011).

In an attempt to study the effects of temperature and relative humidity on the viability of the SARS-CoV, a study found that low temperature and low humidity was able to prolong survival of virus on contaminated surfaces (Chan et al., 2011). The same was found to be true for MERS-CoV. A study reported the stability of MERS-CoV at 20 °C and 40% relative humidity; 30 °C and 30% relative humidity; and 30 °C and 80% relative humidity, and concluded that MERS-CoV was more stable at lower temperature and lower humidity conditions (van Doremalen et al., 2013). In another study, two sets of climatic conditions were used in order to establish the inactivation of MERS-CoV: one represented the common indoor office environment (25 °C and 79% relative humidity) and the other represented the climatic conditions of the Middle Eastern region where the virus outbreak started (38 °C and 24% relative humidity) (Pyankov et al., 2018). Authors found that the virus had a better survival rate at a lower temperature, with virus decay being higher in hot and dry air.

In a recent study, atomic force microscopy was applied to investigate the topographical changes of SARS-CoV-2 virions exposed to high-temperature treatments, reporting that after the treatment, the virus had much fewer less distinct spikes, their trigonal shape not being able to be resolved, suggesting heat-induced inactivation of SARS-CoV-2 (Kiss et al., 2020). Another study reported that the virus was stable at 4 °C in virus transport medium, but sensitive to heat, and that at 22 °C and 65% relative humidity had a negative effect on viral survival on smooth surfaces (Chin et al., 2020). Other studies have reported the effects of humidity and temperature on SARS-CoV-2 transmission based on meteorological data and statistical analysis (Auler et al., 2020; Ma et al., 2020; Méndez-Arriaga, 2020; Meo et al., 2020; Meyer et al., 2020; Sajadi et al., 2020; Ward et al., 2020; Wu et al., 2020; Xie and Zhu, 2020; Yao et al., 2020). Although all of them have reported a correlation between temperature and relative humidity and the number of new COVID-19 cases, there is still some controversy regarding whether one or both variables have a positive, negative or no effect on the number of new cases. The main outcomes of these studies are presented on Table S1 (supplementary material).

Given the genetic and structural similarities between SARS-CoV, MERS-CoV and SARS-CoV-2, one might suggest that higher temperatures and relative humidities could have an impact on the viability of SARS-CoV-2 in the environment. Nonetheless, the effect of these

conditions seem to be residual (Meyer et al., 2020; Wu et al., 2020) considering the increasing number of cases in the south of USA, Brazil and India, where high temperatures and humidities have been observed.

Moreover, it should be noted that although SARS-CoV and MERS-CoV can give us an idea of how SARS-CoV-2 might behave, using SARS-CoV and MERS-CoV to predict the behaviour and spread patterns of SARS-CoV-2 is not advisable, as these three viruses are different, and SARS-CoV-2 might not necessarily follow the same patterns as the aforementioned viruses and more studies are needed in this subject to determine how these environmental variables might impact the virus transmission.

Other factors, such as human mobility patterns and contact rates, should also be taken into consideration as contributing factors to the different transmission rates in different countries (Badr et al., 2020). In developing countries such as Brazil and India, other transmission routes (e.g., the contact and droplet routes) may account for the increasing number of cases rather than the airborne route, as these countries are very densely populated, have overcrowded accommodations and lack of access to basic services, therefore enabling the contact and droplet routes of transmission and spread of the virus. These individual and collective factors such as political, social, economic and cultural conditions should be considered when analyzing the spread of the virus in these countries, as they may play a more significant role than temperature and relative humidity (Auler et al., 2020). Specific measures such as quarantines and lockdowns can also affect the incidence of the virus, as different countries have different approaches and mitigation strategies to deal with the pandemic.

4. Conclusions

Among the reviewed literature, only a few papers explored viral viability in air samples, which is probably due to the difficulty and limitation of many research groups regarding BSL-3 facilities. Nonetheless, efforts should be directed towards the development of novel or adapted analytical methods to reliably and systematically determine the infectivity of SARS-CoV-2 viral particles as this would enable not only routine fundamental insights into the effective spread of the virus and its societal impact, as well as enabling effective biosensing strategies for on-site determination of viral infectivity as previously mentioned.

Currently, there is still debate about whether or not SARS-CoV-2 is transmitted through aerosols produced by infected people during talking, singing sneezing, coughing and breathing, and further studies regarding this route of transmission are needed in order to clarify the extent of aerosol transmission of SARS-CoV-2, as this would have important implications for public health and infection-control policies.

Moreover, higher temperatures and high relative humidity can have an effect on SARS-CoV-2 viability in the environment as reported in previous studies to this date. However, these studies are experimental, and do not support the fact that the virus has efficiently spread in the tropical regions of the globe. In these regions, other transmission routes such as through contact and droplets might be responsible for the majority of the reported cases, along with other factors such as human mobility patterns and contact rates. More studies focusing on seasonality are needed to determine the real impact of the variables temperature and relative humidity on SARS-CoV-2 infection spread, as well as a better comprehension of its transmission mechanisms, especially regarding the airborne route of transmission.

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Author's contribution

PG: Formulation of the research questions and study design, writing of the manuscript, methodology and approval of final version. **MSJN:** Discussion, critical review, revision and approval of final version. **RS:** Discussion, critical review, revision and approval of final version. **SIVS:**

Formulation of the research questions and study design, critical review of the manuscript, methodology and approval of final version. **JRM:** Formulation of the research questions and study design, critical review of the manuscript, methodology, and approval of final version.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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