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Transmission of SARS-CoV-2 in the workplace: Key findings from a rapid review of the literature

Jennie Cox[1], **Brian Christensen**[1], **Nancy Burton**[1], **Kevin H. Dunn**[1], **Mikaela Finnegan**[2], **Ana Ruess**[2], **Cherie Estill**[1]

[1]National Institute for Occupational Safety and Health, Cincinnati, OH, USA

[2]Gryphon Scientific, Takoma Park, MD, USA

Abstract

At the beginning of the COVID-19 pandemic, the primary route of transmission of the SARS-CoV-2 virus was not well understood. Research gathered from other respiratory infectious diseases, including other coronaviruses, was the basis for the initial perceptions for transmission of SARS-CoV-2. To better understand transmission of SARS-CoV-2, a rapid literature review was conducted from literature generated March 19, 2020, through September 23, 2021. 18,616 unique results were identified from literature databases and screened. Of these, 279 key articles were reviewed and abstracted covering critical topics such as environmental/workplace monitoring, sampling and analytical method evaluation, and the ability of the virus to remain intact and infectious during sampling. This paper describes the results of the rapid literature review, which evaluated pathways that contribute to transmission as well as the strengths and limitations of current sampling approaches. This review also evaluates how different factors, including environmental conditions and surface characteristics, could impact the transmission potential of SARS-CoV-2. A continual rapid review in the midst of a pandemic proved particularly useful for quickly understanding the transmission parameters of the virus and enabled us to comprehensively assess literature, respond to workplace questions, and evaluate our understanding as the science evolved. Air and surface sampling with the accompanying analytical methods were not generally effective in recovering SARS-CoV-2 viable virus or RNA in many likely contaminated environments. In light of these findings, the development of validated sampling and analysis methods is critical for determining worker exposure to SARS-CoV-2 and to assess the impact of mitigation efforts.

INTRODUCTION

When the first COVID-19 cases were identified in the U.S., the primary route of exposure was not well understood. Initial perceptions of the potential contributions of different modes of transmission for SARS-CoV-2 were largely informed by research conducted on other respiratory infectious diseases such as SARS-CoV-1, MERS, tuberculosis, and influenza. Based on previous research, it was well understood that humans continuously emit aerosols,

Contact name: Dr. Jennie Cox, Contact phone: 513-458-7140, qxi3@cdc.gov.

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and that, generally, larger particles, such as droplets, tend to travel shorter distances and settle faster than smaller particles (Wells 1934). It was also well known that some respiratory viruses could remain viable in aerosols and surfaces for long periods of time (Boone and Gerba 2007; Tellier et al. 2019). SARS-CoV-1 was considered to be mainly transmitted by a close person-to-person contact through droplets or by contacting contaminated surfaces (Olsen et al. 2003; Otter et al. 2016). This knowledge suggested to researchers and public health practitioners that SARS-CoV-2 would spread through two primary pathways: 1) viral-laden droplets and 2) hand-to-mucus membrane transport of viral particles following direct or indirect contact with a contaminated surface. Moreover, these perceptions influenced what mitigation strategies were prescribed globally, including in the United States. The mitigation strategies during the early stages of the pandemic primarily focused on 1) physical distancing and masking (or source control) to reduce potential exposures to droplets (Eames et al. 2012; Hens et al. 2009; MacIntyre et al. 2016) and 2) cleaning and disinfecting surfaces to address the potential for contact-mediated exposures. Early recommendations to the public included 1) taking steps to protect yourself by cleaning hands often and avoiding close contact, and 2) taking steps to protect others by staying home if sick, covering coughs and sneezes, wearing a facemask in public if sick, and cleaning and disinfecting surfaces.

Transmission can occur when droplets containing an infectious agent are propelled a short distance through the air (less than two meters) and deposited on a person's mucous membranes, or when small particles (e.g., aerosolized droplet nuclei) with viable virus are generated and are inhaled by a susceptible host (Otter et al. 2016; PHAC 2011). The size distribution of exhaled particles is primarily influenced by the mode of exhalation (e.g., normal breathing, coughing, sneezing, etc.). In general, smaller particles are continuously emitted during normal breathing and talking, and 'violent' exhalatory events such as coughing or sneezing emit larger numbers of both large and small particles (Asadi et al. 2019; Johnson et al. 2011). Humans can emit particles ranging from 0.1–500 microns (μm) in diameter; however, most emitted particles by count do not exceed 10 μm in diameter (Gralton et al. 2011; Johnson et al. 2011; Lindsley 2010; Milton et al. 2013). In still air, a 100 μm particle takes 4 seconds to fall 1 meter, while a 10 μm particle takes 5.4 minutes and a 1 μm particle takes 8 hours to settle the same distance (Hinds 1999). Larger droplets generally deposit in the upper respiratory tract, and smaller airborne particles (typically considered < 5 μm) can penetrate the lower respiratory tract and alveolar space, but this size distinction can vary between studies (Kutter et al. 2018). Smaller particles can deposit more deeply into the lungs, can be exhaled and subsequently deposited in the upper respiratory tract, or be fully exhaled, with exhaled particles typically being <1 μm (Tellier 2009; Vincent 2005). A study by Pan et al. (2019) found, when recovering aerosolized MS2 bacteriophages in artificial saliva, an 82% infectious recovery (infectious viruses/total counts) at 0.12 μm, ~50% recovery at 0.09 μm, and very low recovery at 0.06 μm.

Direct contact transmission of respiratory infections would include direct physical contact such as a susceptible host shaking hands with an infected individual's contaminated hands, whereas indirect contact transmission would involve the passive transfer of a virus by contact with an inanimate object, such as a susceptible host touching a door handle contaminated with the virus (PHAC 2011), with delivery of infectious material to a susceptible site such as the nose or mouth. It was well understood, prior to the COVID-19

pandemic, that 1) viruses can remain infectious on surfaces for a long period of time (Bean et al. 1982; Chan et al. 2011), 2) the persistence of viruses on surfaces is partially dependent on the type of surface (porous vs non-porous) on which the virus resides and other material characteristics, such as surface charge and hydrophobicity (Bean et al. 1982; Lai 2005), and 3) external factors, including environmental conditions and the viral suspension medium, can significantly alter the persistence of viruses on surfaces (Chan et al. 2011; Lai 2005; Otter et al. 2016). It was also understood that persistence on surfaces is largely influenced by viral species and strain (Otter et al. 2016). This is well illustrated in reviewing articles that have previously reported on the persistence of respiratory syncytial virus and avian respiratory virus, which remained infectious on surfaces for 6 hours and 6 days, respectively (Hall, Douglas and Geiman 1980; Tiwari et al. 2006). Together, indirect and direct contact transmission have likely played significant roles in spreading infection during viral outbreaks that have taken place in the past (Chen et al. 1989; Guery et al. 2013; Lee et al. 2003; Oh et al. 2015); however, the extent of direct or indirect contact transmission that has taken place during previous viral outbreaks remains poorly understood as attempts to classify outbreaks according to transmission pathway have been severely limited in scope (Kutter et al. 2018).

The collection and analysis of viral samples can generally be split into culture-based and culture-independent methods (Julian et al. 2011; Lindsley et al. 2017; Verreault, Moineau and Duchaine 2008). Polymerase Chain Reaction (PCR) and other culture-independent methods (detection of viral RNA/DNA, enzyme-based assays, immunoassays, electron microscopy) are more widely used than culture-based methods but lack the ability to determine if the virus is infectious. Culture-based methods have the challenge of maintaining the infectivity of a virus during and after collection and culturing host cells for viral replication. For surface sampling, swabs are commonly utilized for culture and culture-independent studies, and for aerosol sampling, impingers are the most commonly used for culture-based analysis (Julian et al. 2011; Verreault, Moineau and Duchaine 2008). For culture-independent analysis, filter and cyclone-based aerosol samplers are often used due to their simplicity and effectiveness at collecting aerosol particles of all sizes (Lindsley et al. 2017).

The National Institute for Occupational Safety and Health (NIOSH) established the Disaster Science Responder Research (DSRR) Program to develop an approach that allows for timely and scalable research that can be implemented before, during, and after a public health emergency (NIOSH 2021). The DSRR strategic goals include identifying critical topic areas to enhance safety and health among all workers. A rapid literature review was conducted biweekly within these critical topic areas from June 22, 2020, to September 23, 2021. One goal within the Occupational Environment and Exposure Assessment topic area was to better understand how different modes of transmission contribute to the spread of SARS-CoV-2. This paper describes the results of the rapid literature review for this topic area, which attempted to evaluate the pathways that contribute to transmission, to what extent, and the strengths and limitations of current sampling approaches in assessing the risk of SARS-CoV-2 exposure and transmission. This review will also evaluate the validity of current sampling approaches (surface, wastewater, and air) in characterizing pathway-specific transmission risk, as well as evaluate how different factors, including

environmental conditions and surface characteristics, could impact the transmission potential of SARS-CoV-2.

METHODS

As research efforts during the COVID-19 pandemic progressed, the amount of literature related to COVID-19 rapidly increased. Subject matter experts independently searched and reviewed research articles via PubMed, Google Scholar, and other search engines. In addition to subject matter expert paper selection, a rapid review methodology was used to ensure that the most novel and robust data were available on an ongoing basis.

Literature searches were performed on a bimonthly basis to continuously identify research on COVID-19 risks for workers, particularly risk of infection via aerosols, contaminated surfaces and through exposure to bodily fluids that can contaminate the workplace. The review described here was a part of a larger rapid review and accounts for the specific topic area “Occupational Environment and Exposure Assessment.” Articles with different search parameters could also have been placed into eight additional critical topic areas including Economics, Engineering Controls, Epidemiology and Surveillance, Mental Health, Occupational Violence, Personal Protective Equipment, Transmission and Occupational Health, and Zoonosis. Articles were typically only placed in one research topic area to reduce redundancy of review efforts.

1.0 Search Strategy

Literature searches of publications (peer reviewed and not yet peer reviewed) were conducted every two weeks from June 22, 2020, to October 8, 2020, using a COVID-19 literature library maintained by the Centers for Disease Control and Prevention (CDC), at which point the CDC migrated its library to the World Health Organization’s (WHO) COVID-19 Global Research Database (WHO 2020). Literature searches were conducted in the WHO library once every two weeks until September 23, 2021, using the search strings listed in the Supplemental Information. Additional publications were identified through other literature reviews and the Department of Homeland Security’s Master Question List for COVID-19 (caused by SARS-CoV-2)(DHS 2022).

2.0 Inclusion and Exclusion Criteria

All literature on SARS-CoV-2, published in English between March 19, 2020 (earliest upload date in the CDC literature library) and September 23, 2021, were included, including preprints and other non-peer-reviewed publications irrespective of where the study occurred. Original articles were prioritized, but research letters and reviews were also included. Studies performed in all types of workplaces were included (e.g., hospitals, dental settings, restaurants, manufacturing plants, etc.). Studies that conducted sampling exclusively in homes of patients were also included to capture exposure risks to home healthcare workers. Any literature not relevant to workplace exposure and safety was excluded.

3.0 Article Screening

Results were screened first by title, and those that remained were screened by abstract. For each publication screened, a researcher created a summary noting the key take-away findings of the study, study limitations, and additional relevant information included in the publication. For publications with novel data or important findings, a shortened version of the summary was written and added to a condensed report. As preprints were subsequently published, the summaries and condensed reports were updated, and the citations revised.

RESULTS

18,616 unique results were identified from the literature databases and screened (data not shown). Of these, 279 key articles were reviewed and abstracted, in which 40 were non-peer-reviewed preprints, while the rest were peer-reviewed publications (Supplemental Table S1). Key articles were determined by subject matter experts as those articles that will move forward the science of SARS-CoV-2, have sound methods and conclusions, and inform actionable decisions. Articles reviewed and abstracted covered a range of topics, but can be generally categorized into exposure-route focused topic areas below:

- Environmental/Workplace Monitoring: Largely environmental sample collection from healthcare settings or public spaces, including bodily fluids, surface swab samples, aerosol samples, and other types of samples (mostly wastewater) (Table 1).
- Sampling/Analytical Method Evaluation: Studies that compared various sampling methods, such as aerosol samplers or transport media for surface swabs (Table 1).
- Viral Persistence of Infection/Decay: Experimental laboratory studies, such as inoculating various surfaces with SARS-CoV-2 and assessing half-life (Table 1).
- Literature reviews: Studies that collected journal articles to draw conclusions from the collated dataset (Table 2).
- Epidemiology: Includes contact tracing and outbreak reports, such as analyses of superspreader events (Table 2).
- Exposure Modeling: Includes mathematical models, computational fluid dynamic models, aerosol particle models, etc. (Table 2).

The summaries of the reference type and topic area for the number of citations are included in Tables 1 and 2. Articles found by subject matter experts and those that fell into other critical topic areas are also accounted for in Table 2. The 279 articles that were reviewed and abstracted are listed in Supplemental Table 1 with their associated reference type and topic area. The accumulation of information was divided into an overview of bodily fluids, transmission pathways including aerosol and surfaces, and general methods evaluating RNA and viability. The authors utilized representative manuscripts from the key articles in order to summarize the state of the science for this manuscript.

1.0 Bodily Fluids

Since biological fluids can serve as potential sources of exposure in the workplace, several studies have been conducted both in the field and the laboratory to determine the presence and persistence of infectivity of SARS-CoV-2 in these fluids.

Respiratory tract secretions such as mucus, sputum and saliva are biological fluids that can contain viral material and make up respiratory droplets that are released into the air. Viable SARS-CoV-2 was isolated from viral cultures of upper respiratory tract specimens from infected individuals in two studies (Bullard et al. 2020; Wolfel et al. 2020). SARS-CoV-2 viral RNA was not found in sweat samples collected from hospitalized COVID-19 patients (Arslan et al. 2021). One study did not detect SARS-CoV-2 viral RNA in urine, semen, and expressed prostatic secretion samples from recovered COVID-19 male patients (Ruan et al. 2021), but two additional studies evaluated urine for viable SARS-CoV-2 (infectious) virus, and one found viable virus (Kashi et al. 2020). SARS-CoV-2 RNA was not found in vaginal fluid or cervical exfoliated cell and was only found in 1 anal swab sample from 27 women with positive SARS-CoV-2 RNA respiratory samples (Cui et al. 2020). SARS-CoV-2 viral RNA was found in fecal samples of 6 of 8 COVID-19 hospital patients and in the urine of 1 of 8 patients. Vero E6 cells were inoculated with fecal samples and showed no cytopathic effects indicating a lack of infectivity (Albert et al. 2021).

Studies looking at tears and ocular secretions have had mixed results. Several studies conducted conjunctival swab testing of COVID-19 patients using SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) analysis (Mohaqiq et al. 2021). Out of nine studies, investigators in three studies did not detect SARS-CoV-2 RNA. In the other six studies, investigators found that a combined 5/131 samples from 3/52 known COVID-19 patients were positive for SARS-CoV-2 RNA (Mohaqiq et al. 2021). A case report showed that an ocular sample from a patient with conjunctival hyperemia detected SARS-CoV-2 RNA and was tested for viable virus with Vero E6 cells in which a cytopathic effect was observed 5-days post-inoculum (Colavita et al. 2020).

There have been several laboratory-based studies looking at the persistence of infectivity of the virus in various bodily fluids. A study tested SARS-CoV-2 persistence of infectivity in suspensions of human feces and urine. Each sample was inoculated with 0.3 milliliter of virus stock with an infectious titer of 10^6 50% tissue culture infectious dose (TCID50). This study showed viable SARS-CoV-2 virus was recoverable for up to 48 hours in feces and 3-4 days in urine (Liu et al. 2021b). A study using 10^6 surrogate viral particles found, after contaminating a toilet bowl rim, toilet seat top, and toilet seat underside prior to flushing, $<10^3$ particles could be found on the seat, handle, floor, and walls via dispersal and deposition following flushing (Sassi et al. 2018). SARS-CoV-2 has been shown to remain viable in both highly acidic and highly basic conditions (Sun et al. 2020).

Investigators added SARS CoV-2 to commercially available human nasal mucus and sputum and assessed persistence of infectivity under three environmental conditions - 4°C/40% relative humidity (RH), 21°C/40% RH, and 27°C/85% RH (Matson et al. 2020). Fifty μL of prepared solution containing $1x10^5$ TCID50/mL was added to either sealed tubes or onto polypropylene disks and sampled at specific time points. SARS-CoV-2 persistence of

infectivity was greater in liquid droplets than when dried on surfaces using human nasal mucus and sputum. Lower temperature and lower relative humidity were associated with longer half-lives, but no virus survived more than 48 hours. They found viral RNA for up to a week in all samples despite the absence of viable virus (Matson et al. 2020). SARS-CoV-2 stability was tested in nasal mucus, sputum, saliva, tears, feces, urine, breast milk, blood, and semen. Viral stability was assessed by inoculating Vero E6 cells and recovering infectious virus at various time points. Samples were tested in liquid (sealed tubes) and on stainless steel surfaces (except human urine, which was only tested in liquid). The virus was stable for up to 21 days in nasal mucus, sputum, saliva, tear, urine, blood, and semen; it remained infectious significantly longer under winter (5°C and 75% RH) and spring/fall conditions (13°C and 66% RH) than under summer conditions (25°C and 70% RH). The virus was stable up to 24 hours in feces and breast milk (Kwon, Gaudreault and Richt 2021).

One of the additional occupational exposure concerns from bodily fluids was the potential for exposure to their presence in wastewater. RT-PCR testing for SARS-CoV-2 RNA in wastewater was commonly used in multiple countries but did not look at infectivity for the virus (Anand et al. 2022; Baldovin et al. 2021; Miyani et al. 2020; Tomasino et al. 2021). A review of published studies looked at the risk of occupational exposure to SARS-CoV-2 in wastewater residuals and biosolids. Although SARS-CoV-2 RNA can be detected in wastewater, it was noted that few studies have been able to isolate infectious virus in fresh feces (Jeong et al. 2020; Liu et al. 2021b; Xiao et al. 2020). As wastewater goes through the various treatment processes, the amount of infectious virus is expected to decrease similarly to other viral pathogens. The recommendations to reduce exposures to other pathogens should also be effective to reduce the risks of SARS-COV-2 infections (Brisolara et al. 2021).

2.0 Aerosol

2.1 Aerosol transmission

2.1.1 Aerosol as a source: Several researchers have encouraged a stronger acknowledgement of the role of aerosol transmission with particles < 5 μm for SARS-CoV-2 (Allen and Marr 2020; Morawska and Cao 2020; Prather et al. 2020). A group of experts from the fields of aerosol science, ventilation, engineering, physics, virology and clinical medicine reviewed the evidence for airborne transmission mechanisms and concluded that small airborne particles are potentially more likely to lead to infection via inhalation of airborne viruses than by contact or large droplet mechanisms (Tang et al. 2021). The potential for transmission of SARS-CoV-2 includes the spread of virus-containing aerosolized particles from normal exhalatory activities such as breathing, speaking, singing, coughing, or sneezing, as well as through virus-containing large respiratory droplets that fall to the ground more quickly (CDC 2021; Chen et al. 2021; Coleman et al. 2021; Schijven et al. 2021; Stadnytskyi et al. 2020). As smaller particles capable of inhalational transmission have become a significant concern in the transmission of SARS-CoV-2, studies have focused on aerosol concentrations while breathing, speaking, and singing, as well as when wearing masks (Adenaiye et al. 2021; Buonanno, Stabile and Morawska 2020; Chen et al. 2021; Coleman et al. 2021; Grinshpun and Yermakov 2021).

The viral load expelled from an infectious person at any given time is primarily dependent on three factors, 1) the length of time since the onset of the illness, 2) the severity of the illness, and 3) physiological factors that govern the emission rate of aerosols and therefore viral particles. In a study of the exhaled breath particles of eight nonhuman primates infected by aerosol with SARS-CoV-2 and 194 healthy human subjects, Edwards et al. found that the number of exhaled aerosol particles vary between subjects by three orders of magnitude, with exhaled respiratory droplet number increasing with degree of SARS-CoV-2 infection and elevated body mass index and age (Edwards et al. 2021). Overall, the emission rate of exhaled aerosol particles began to increase on day three post-infection and continued to increase through day seven (Edwards et al. 2021). It was also observed that 18% of human subjects (35) accounted for 80% of the exhaled bioaerosol of the group (194), reflecting a distribution of bioaerosol analogous to a classical 20:80 superspreader of infection distribution (Edwards et al. 2021). Studies of exhaled aerosols from SARS-CoV-2 positive patients have shown that a large portion of viral RNA (between 60–90%) is contained in respirable-sized particles (less than 5 μm in size)(Coleman et al. 2021; Santarpia et al. 2021). Currently, an accurate quantitative estimate of the infective dose of SARS-CoV-2 in humans is not feasible and needs further research, but a review by Karimzadeh et al. estimated an infectious dose could be as small as 100 plaque forming units (Karimzadeh, Bhopal and Tien 2021). A SARS-CoV-2 infection has lower morbidity and mortality, but greater infectivity, compared with SARS-CoV-1 and MERS (Peeri et al. 2020).

Being in an enclosed or crowded space with an infected individual also impacts transmission, as viable aerosolized SARS-CoV-2 can persist in the air. Using laser light scattering to visually capture how exhaled particles behave during normal speech, a laboratory study found that particles remained suspended for 8–14 minutes in stagnant air, based on the weighted average decay rate and half-life within the enclosure. The calculated terminal velocity corresponds to droplet nuclei of ~ 4 μm diameter, or 12- to 21-μm droplets prior to dehydration (23°C and RH 27%)(Stadnytskyi et al. 2020). SARS-CoV-2 viable virus was isolated in aerosols 3 hours after aerosolization in one lab study (21–23°C, 65% RH)(van Doremalen et al. 2020) and up to 16 hours in aerosols in another lab study (~23°C, ~54% RH)(Fears et al. 2020).

Several laboratory and modeling studies have assessed the influence of environmental conditions (temperature, relative humidity, and sunlight) on the viability of SARS-CoV-2. Relative humidity could affect transmission, as modeling studies have shown that droplets quickly evaporate (reducing particle size) and, therefore, stay in the air longer (Dombrovsky et al. 2020; Netz and Eaton 2020; Smither et al. 2020). For these particles, their airborne lifetime is related to the turnover time of the air handling system which could become the primary driver of concentration of evaporated droplets (Netz and Eaton 2020). Low temperature (10°C, 50°F) and high humidity (80%) were both associated with longer droplet lifetimes, by slowing the evaporation of microdroplets and contributing to the long-term survival of the airborne virus (Dombrovsky et al. 2020). However, other studies have shown that relative humidity alone (<88%) does not affect the infectious decay rate of the airborne SARS-CoV-2 (Schuit et al. 2021; Schuit et al. 2020; Smither et al. 2020). Simulated sunlight (ultraviolet [UV] 280–400 nm) rapidly inactivates (90% loss in 6 min at summer sunlight conditions) SARS-CoV-2 in aerosols (Schuit et al. 2020). A study looking at a single

particle size distribution representing breathing/talking (2 μm mass median aerosol diameter) reported that increased temperature and simulated sunlight have a greater influence on decay of SARS-CoV-2 than humidity across the range of conditions tested (Dabisch et al. 2021).

When comparing SARS-CoV-2 and SAR-CoV-1 in aerosols, one study found no significant difference in survival under the conditions tested (van Doremalen et al. 2020). The stability of SARS-CoV-2 (CA_CDC_5574/2020, an isolate of the B.1.1.7 lineage) in aerosols did not vary greatly from three earlier SARS-CoV-2 isolates (WA-1/2020, NY-PV08449, and France/IDF0372/2020), which suggested that the increased transmissibility associated with later SARS-CoV-2 lineages was not due to enhanced survival in the environment (Schuit et al. 2021). Another study found that the aerosol efficiency (i.e., ratio of nebulizer concentration to aerosol concentration) of SARS-CoV-2 surpassed those of SARS-CoV-1 and MERS, suggesting retained infectivity and virion integrity for up to 16 hours in respirable-sized aerosols (Fears et al. 2020).

2.1.2 Aerosol epidemiological studies: Examples of superspreader events: Airborne transmission, particularly in closed and poorly ventilated spaces with crowds, likely contributes to some “superspreading” events, as observed in several reported cases. These include a choir practice in Skagit Valley, WA, where 61 members congregated for their weekly rehearsal in a county where only 7 confirmed COVID-19 cases had been previously reported at that time (Hamner 2020). The choir members reportedly avoided physical contact as well (such as hugs and handshakes). Among the persons who attended the choir practice at which one person was known to be symptomatic, 53 cases were identified, including 33 confirmed and 20 probable cases. Researchers investigating this outbreak have concluded that it is unlikely contact transmission due to the high attack rate of between 53–87% (based on confirmed or confirmed and probable cases)(Miller et al. 2021).

Other superspreader events have also been reported which may indicate short-range aerosol transmission. A call center in South Korea saw a transmission rate of 47% with most cases being located on one side of one floor of the building (Park et al. 2020). This outbreak highlights several factors that are associated with potential airborne transmission including indoor space, crowding, and talking/vocalizing (aerosol generating activity). A SARS-CoV-2 outbreak in two meat processing plants in Germany showed workers as far as 8 m (26 ft) were likely infected with the same strain from the index case. The authors suggest airborne transmission because of the poor ventilation (<1 air change per hour). In addition, the work areas were kept at <10°C which could contribute to the slower evaporation of droplets (Günther et al. 2020). Of 217 adult students who participated in high-intensity aerobic dance classes across 12 facilities, 57 (26%) were infected with SARS-CoV-2 from contact with instructors. Characteristics that might have led to transmission from the instructors included large class sizes, small spaces, and intensity of the workouts (Jang, Han and Rhee 2020). An investigation of a bus superspreader event in eastern China showed that of two buses which carried the index patient, only riders on one bus developed COVID-19 symptoms (24 of 68). Despite potential opportunities for exposure at a worship service and a luncheon, none of the riders on the other bus developed symptoms, suggesting disease transmission occurred during the ride, likely as a result of airborne transmission (Shen et al. 2020). Finally, an outbreak of COVD-19 affected 10 persons from 3 families who had eaten at the same air-

conditioned restaurant in Guangzhou, China (Lu et al. 2020). An in-depth ventilation study conducted at the restaurant found that poor ventilation (minimal outside air introduction), and problematic recirculatory airflows from a ceiling mounted air conditioner likely allowed the short-range transmission of infectious aerosols from patrons at one table to another up to a distance of 4.6 m from the index patient (Li et al. 2021). The authors concluded that "extended short-range aerosol transmission of the virus is possible in crowded and poorly ventilated enclosures." In addition, video tapes that captured the outbreak event showed that direct or indirect contact (via plates or silverware) between the index case and individuals sitting at other tables did not occur, thus providing further confirmation that contact transmission likely did not play a significant role during the exposure event (Zhang et al. 2021).

2.2 Aerosol sampling

2.2.1 Examples of aerosol sampling in healthcare: To attempt to find SARS-CoV-2 in the air, a large number of studies focused on healthcare facilities with COVID-19 positive patients (Aghalari et al. 2021; Birgand et al. 2020; Rahmani et al. 2020). Birgand et al. (2020) assessed 24 studies of air contamination in hospital settings for a total of 893 air samples. Overall, 82 of 471 air samples (17.4%) from close patient environments (i.e., patient rooms or bays) were positive for SARS-CoV-2 RNA. The positivity rate was 5 of 21 air samples (23.8%) in toilets, 20 of 237 (8.4%) in clinical areas, 15 of 122 (12.3%) in staff areas, and 14 of 42 (33.3%) in public areas. A total of 81 viral cultures were performed across 5 studies, and 7 (8.6%) from 2 studies were positive, all from close patient environments (2 to 4.8 m away from the patients) (Figure 1). Rooms for the removal of protective apparel and patient rooms had high concentrations of SARS-CoV-2 (varying from 0.9×10^3 to 40×10^3 copies/m^3 and 3.8×10^3 to 7.2×10^3 TCID50/m^3)(Birgand et al. 2020). It is clear that while there are many studies which found aerosol SARS-CoV-2 RNA (Dumont-Leblond et al. 2020; Guo et al. 2020; Lednicky et al. 2020; Mallach et al. 2021; Santarpia et al. 2020) and that many were somewhat limited in or unable to find SARS-CoV-2 RNA (Cheng et al. 2020; Lane et al. 2021; Li et al. 2020; Wei et al. 2020a; Zhou et al. 2020), only a few found viable SARS-CoV-2 in air samples (Lednicky et al. 2020; Santarpia et al. 2021).

2.2.2 Methods for collecting airborne SARS-CoV: A variety of aerosol sampling equipment and methods have been used in field-based studies in an attempt to capture SARS-CoV-2 in the air, including but not limited to, the SKC personal button sampler (Santarpia et al. 2020), NIOSH BC251 (Chia et al. 2020), miniature cascade impactor (Liu et al. 2021b), SASS 2300 wetted wall cyclone (Guo et al. 2020), Sartorius MD8 (Liu et al. 2021b; Santarpia et al. 2020), Coriolis μm air sampler (Moore et al. 2021), an AV1000 sampler (Hadei et al. 2021), the VIVAS (Viable Virus Aerosol Sampler) and its commercial version BioSpot-VIVAS BSS300P (Lednicky et al. 2020). The VIVAS and the Bio-Spot VIVAS utilize a water vapor condensation mechanism, which facilitates the likelihood of isolating the virus in tissue culture (Lednicky et al. 2020). In a laboratory setting, Ratnesar-Shumate et al. (2021) evaluated the performance of eight aerosol sampling devices for collection and the preservation of infectivity of airborne SARS-CoV-2. Similar concentrations of infectious SARS-CoV-2 were measured in aerosols for the majority of

the samplers tested (all-glass impinger, Biosampler, Sioutas impactor, gelatin filter, PTFE filter and NIOSH BC 251), with the exception of the midget impingers (all-glass midget impinger and PFA (fluoropolymer) midget impinger), which measured significantly lower concentrations of SARS-CoV-2. This study potentially could assist in interpretation of the SARS-CoV-2 viral loads in air samples and help inform sampling device selection in future studies (Ratnesar-Shumate et al. 2021).

3.0 Surfaces

3.1 Surfaces as a source—The importance of indirect contact transmission in spreading COVID-19 infection has been methodically explored. For most studies that have evaluated surface contamination, the viral load of SARS-CoV-2 on positive surface samples has been low suggesting that contaminated surfaces pose a minimal risk for COVID-19 transmission (Kampf, Lemmen and Suchomel 2021); however, depending on the materials and processes used, some methods could underestimate the true concentration of SARS-CoV-2 on surfaces. Viral losses that occur during sample collection, transport, and extraction of SARS-CoV-2 RNA prior to quantification likely lead to an underestimation of the risk of contact transmission (Parker et al. 2020). Other parameters beyond viral load at the time of contact also require consideration. One modeling study reported a wide range of transmission probabilities based on an individual's frequency of contact with contaminated surfaces, frequency of contact with mucosal membranes (mouth, nose, eyes), and how efficiently SARS-CoV-2 transferred from surface-to-hand and from hand-to-mucosal membranes (mouth, nose, eyes)(Pitol and Julian 2021). Another modeling study reported a significant difference in the probability of contact transmission based on whether the mouth or nose was touched after contact with a contaminated surface (Furuya 2020).

Factors that could influence the extent of SARS-CoV-2 contamination prior to an exposure event(s) include the initial viral titer on the surface, the decay rate of the virus on that surface, and the duration of time that has elapsed since the surface was initially contaminated. However, one study has shown that the rate of SARS-CoV-2 viral decay on a surface is independent of the initial viral titer (Paton et al. 2021). Several experimental studies have reported that higher temperature, UV intensity, and UV duration are associated with increased SARS-CoV-2 decay rate (Heilingloh et al. 2020; Inagaki et al. 2020; Ratnesar-Shumate et al. 2020; Riddell et al. 2020; Sagripanti and Lytle 2020; Simmons, Carrion and Alfson 2020; Yap et al. 2020). Additionally, high temperatures paired with low humidity can boost the disinfecting power of UV radiation by speeding up the rate of desiccation of particles on surfaces containing SARS-CoV-2 (Nicastro et al. 2021). In the absence of UV radiation, higher relative humidity is associated with a faster SARS-CoV-2 decay rate (Biryukov et al. 2020); however, it remains unclear as to whether influence of relative humidity on viral decay rate is independent of temperature (Biryukov et al. 2020; Chan et al. 2011; Guillier et al. ; Matson et al. 2020).

Aside from environmental factors, surface type also plays a role in the decay rate of SARS-CoV-2. Several experimental studies have reported a positive association between surface porosity and viral decay rate with highly porous surfaces such as cotton increasing the viral decay rate substantially, most notably within the initial hour following surface contamination

(Chin et al. 2020; Harbourt et al. 2020; Kasloff et al. 2021; Liu et al. 2021a; van Doremalen et al. 2020). Supplemental Table S2 illustrates how surface type, temperature, and relative humidity impact the decay rate of SARS-CoV-2. Additionally, the impacts of surface porosity were further demonstrated in an exposure model study that evaluated the risk of infection after contact with porous and non-porous surfaces that were inoculated with SARS-CoV-2 (Furuya 2020). Using the experimental exposure model, Furuya et al (2020) estimated that the probability of infection from hand-to-mouth transmission is 2 orders of magnitude higher for non-porous surfaces than porous surfaces. An experimental study that evaluated the impact of surface type and droplet size on SARS-CoV-2 persistence of infectivity reported that droplet size plays an insignificant role in the rate of viral decay (Biryukov et al. 2020); however, the effects of droplet size were only evaluated for non-porous surfaces. Furthermore, while the decay rate of SARS-CoV-2 was recorded for different non-porous surfaces, the drying time for different droplet sizes on each surface was not reported. Chatterjee et al. (2021) experimentally determined that respiratory droplets deposited on porous surfaces adhere to different evaporation mechanisms than droplets deposited on non-porous surfaces. It should be noted, that droplet composition is also a factor, as Pastorino et al. (2020) demonstrated that SARS-CoV-2 infectivity was preserved in the presence of proteins, regardless of the type of surface. Moreover, the interplay between surface porosity, droplet size, droplet composition, and evaporation time requires further evaluation to determine the impact of droplet SARS-CoV-2 persistence of infectivity on surfaces.

3.2 Surface Sampling

3.2.1 Examples of surface sampling in healthcare settings: Due to the heightened potential for COVID-19 transmission in settings where a high density of COVID-19 cases is present, much of the SARS-CoV-2 sampling literature has been dedicated to quantifying levels of SARS-CoV-2 across various matrices, including surfaces, in healthcare settings. While several studies have reported surface contamination in healthcare settings, the frequency of detection has been low (<27%) for most studies (Figure 1)(Kampf et al. 2021). When collecting samples taken directly from different body parts of COVID-19 patients post-mortem, Schroder et al detected SARS-CoV-2 RNA in ~73% of collected samples; however, only 33 decedents were evaluated (Schröder et al. 2021). Across over 25 studies that have collected at least 100 surface samples in healthcare settings, only two studies have detected SARS-CoV-2 RNA in more than 30% of collected samples (Kim et al. 2020; Wei et al. 2020a).

Upon reviewing the literature, a few trends become evident. Samples collected in hospital rooms near the COVID-19 patient (bedrail, pillows, hospital gown, floor near bed, etc.) were more likely to have detectable levels of SARS-CoV-2 RNA (Tan et al. 2020; Wei et al. 2020a). Frequently touched surfaces such as personal protective equipment (PPE), keyboards, hand sanitizer dispensers, and computer mouses also exhibited elevated rates of detectable levels of SARS-CoV-2 RNA (Elbadawy et al. 2021; Wu et al. 2020). SARS-CoV-2 RNA detection on bathroom surfaces such as paper towel dispensers, toilet bowls, toilet flushers, and door handles was commonly reported across studies that evaluated the presence of SARS-CoV-2 in the bathrooms of patients (Dancer et al. 2021). Outside

of experimental settings, no in situ peer-reviewed studies have evaluated the rate of surface contamination before and after routine disinfection; however, one hospital study has evaluated the rate of SARS-CoV-2 RNA across different hospital surfaces before and after the implementation of more thorough disinfectant procedures. Trend analysis demonstrated a significant reduction in the rate of samples that were positive for SARS-CoV-2 RNA across 3 sampling time periods: 1) directly following routine cleaning, 2) 2-weeks after quaternary ammonium-based disinfectant was substituted for detergent, and 3) one-week after a more thorough and frequent cleaning protocol was introduced, which included the intermittent usage of a 2,500 ppm sodium hypochlorite solution (Redmond et al. 2021).

From the subset of surface sampling studies that attempted to evaluate SARS-CoV-2 infectivity, only two peer-reviewed studies have isolated viable virus from a surface sample. SARS-CoV-2 was successfully cultured from multiple surface samples (n=6) collected from an ICU room where a patient was receiving high flow oxygen therapy via nasal cannula (Ahn et al. 2020). Viable virus was only isolated from surface samples collected in close proximity to the patient (<6 ft)(Ahn et al. 2020). Another study was able to culture virus from a surgical mask that was worn by a COVID-19 patient in critical condition (Hu et al. 2020). Virus could only be cultured from one of the nine masks that were positive for SARS-CoV-2 RNA collected from COVID-19 patients who had a range of health conditions, from critically to severely to mildly ill (Hu et al. 2020).

3.2.2. Examples of Surface Sampling in Other Indoor Environments: Few studies have evaluated surface contamination of SARS-CoV-2 outside of healthcare settings. SARS-CoV-2 RNA was detected frequently in one study that collected samples from public transportation bus support bars and handrails (23/58 were positive)(Moreno et al. 2021); however, samples collected from community settings outside of public transportation have infrequently yielded detectable levels of SARS-CoV-2 (Harvey et al. 2020; Karami et al. 2021; Luo et al. 2020; Montagna et al. 2021). Additionally, studies that have evaluated surface contamination in schools and supermarkets have also reported infrequent detection of SARS-CoV-2 RNA from surface samples (<4% of samples across 1 study that sampled school surfaces and 2 studies that sampled supermarket surfaces (Caggiano et al. 2021; Crowe et al. 2021; Singh et al. 2021). From the literature that was reviewed, no surface sampling studies conducted outside of healthcare settings have successfully cultured SARS-CoV-2 from a positive surface sample.

4.0 Methods: PCR and Culturing

Downstream processing of air and surface samples to test for SARS-CoV-2 RNA or viability are similar and considerations for these methods are largely the same. As a part of a laboratory study, Tastanova et al. (2021) evaluated 6 different in-house and commercial RT-PCR detection methods and determined that each method demonstrated similar limits of detection (1 or 2 viral copies/μL), sensitivities (93.6% to 97.8%), and specificities (98.7% to 100%). The RT-PCR results were given in terms of a cycle threshold (Ct) or quantification cycle (Cq) (Bustin et al. 2009), which is the number of amplification cycles needed for detection of the nucleic acid target during the amplification process, and therefore has an association with the amount of viral genetic material in the sample. Tastanova et al.

(2021) also used an external quality assessment quantitative test sample and linear regression modeling to determine a Ct value of 40 as the maximum value a sample is considered positive, and a value of 40 has been utilized by many studies as a method limit of detection (Jin et al. 2021; Lei et al. 2020; Zhou et al. 2020). A study by Zhou et al. (2020) found that Ct values of >30, corresponding to an envelope gene copy number of $<10^5$/mL, are unlikely to be culturable, however, SARS-CoV-2 has been isolated above this threshold (Lednicky et al. 2020; Lednicky et al. 2021).

Most studies have utilized culture media such as Dulbecco's Modified Eagle's Medium (DMEM) or Eagle's Minimum Essential Medium (EMEM) plus bovine serum albumin (BSA), fetal bovine serum (FBS), or a combination of both to mimic the protein content present in clinically relevant media such as saliva, mucus, or sputum. A cross-comparison of the SARS-CoV-2 decay rate across studies that utilized similar experimental conditions (≈ temperature and RH), but different suspension media, reported a significant reduction in the decay rate when SARS-CoV-2 was suspended in nasal mucus or sputum rather than culture medium (Matson et al. 2020; van Doremalen et al. 2020). A separate study that compared the decay rate of SARS-CoV-2 with and without BSA found that the decay rate increased significantly in the presence of BSA (Pastorino et al. 2020). These findings would appear to be contradictory as the inclusion of BSA, used to mimic the protein content of respiratory fluids such as saliva, functioned as a preservative in the Pastorino study (Pastorino et al. 2020); however, respiratory fluids also contain enzymes and mucins that may impede the persistence of infectivity of SARS-CoV-2. Vero E6 (African Green monkey kidney) cells were used to culture virus from air and environmental samples in many viability studies and was the dominant method during the time of this review (Chia et al. 2020; Dumont-Leblond et al. 2021; Dumont-Leblond et al. 2020; Lane et al. 2021; Lednicky et al. 2020; Lednicky et al. 2021; Ratnesar-Shumate et al. 2021; Zhou et al. 2020).

DISCUSSION

At the beginning of the COVID-19 pandemic the WHO focused on two pathways for healthcare workers that included droplet and fomite-mediated transmission for suspected COVID-19 patients, unless one was performing aerosol generating procedures, whereas the CDC recommended a more precautionary approach of N95s for workers (Bahl et al. 2020; Tang et al. 2021). Several studies have emphasized the importance of disinfection practices in combating COVID-19 transmission, particularly in healthcare studies where COVID-19 cases are present, while other studies have suggested that resources would be better allocated towards mitigating airborne-mediated transmission. Utilizing the knowledge gained from epidemiological and laboratory studies has provided insight into transmission of SARS-CoV-2 and enables researchers and policy makers to focus on appropriate mitigation strategies.

Understanding Transmission

Studies have established that SARS-CoV-2 is viable in a variety of bodily fluids including feces, urine and potentially tears. The virus can remain stable in fluids (mucus, sputum, tears, blood, semen) for weeks with infectiousness lasting significantly longer under winter

and spring/fall conditions than under summer conditions. The virus can remain stable in feces for up to 1 day. Knowing the stability of the virus in fluids is essential in directing the efforts for cleaning and disinfection protocols necessary to eliminate infectious material. Similarly, wastewater may be a useful tool for tracking trends for SARS-CoV-2 RNA, but only a few studies were able to isolate infectious virus in fresh feces.

The duration, proximity, and frequency of contact with someone infected with SARS-CoV-2 plays a role in dictating the risk of infection. The vast majority of documented SARS-CoV-2 transmission is associated with close contact in the home or in indoor spaces for a prolonged period of time. SARS-CoV-2 RNA was commonly detected in hospital bathrooms of COVID-19 patients' surfaces such as paper towel dispensers, toilet bowls, toilet flushers, and door handles. Bus handrails and support bars have also detected SARS-CoV-2 RNA, but other public settings such as schools or supermarkets had infrequent detection. Indirect contact transmission is possible but is less likely as it requires an intermediate step for transmission to occur. Simply contacting a contaminated surface before touching the mouth, eyes, or nose does not guarantee transmission, as the viral load of SARS-CoV-2 on a surface at the point of contact and the transfer efficiency of the virus from hand to mucous membrane will ultimately determine the risk of transmission. Therefore, if the viral load is low, then the likelihood of infection will also be low. The results from persistence of infectivity studies of SARS-CoV-2 on surfaces were similar to other studies of other respiratory viruses, such as SARS-CoV-1. Several studies suggested a biphasic decay rate with rapid decay during the initial hour and a slower decay rate thereafter. Additionally, reduced survival rates were seen in more porous materials. These findings suggest that porous surfaces have a reduced viral infectivity particularly after the initial hour of contamination. As the scientific community begins to collectively agree on the dominant transmission pathways of this virus, approaches to disrupt these pathways should help to reduce the spread.

Aerosols have become a significant concern in the transmission of SARS-CoV-2 as studies have found exhaled particles when breathing, speaking and singing will transmit respiratory droplets that can stay suspended in the air. In addition, the number of exhaled particles of an individual post-infection increased day 3 thru 7, which provides a timeframe when transmission could increase. However, other studies have found that the transmission potential was greatest in the first 2 days before and 3 days after onset of symptoms in the index patient (Ge et al. 2021). No difference was typically seen in survival between aerosol SARS-CoV-1 and SARS-CoV-2 or between different aerosol SARS-CoV-2 isolates, but one study found higher aerosol efficiency (i.e., ratio of nebulizer concentration to aerosol concentration) with SARS-CoV-2 which suggested better infectivity and virion integrity compared to those of SARS-CoV-1 or MERS. Some studies have shown that small aerosols can contain significant amounts of viral material and can stay aloft and viable for minutes to hours. As of May 2021, the CDC updated the modes of SARS-CoV-2 transmission to be categorized as inhalation of virus, deposition of virus on exposed mucous membranes, and touching mucous membranes with soiled hands contaminated with virus (CDC 2021). Epidemiological studies evaluating superspreader events, such as the Guangzhou restaurant outbreak, provided evidence that airborne transmission was a likely pathway (Hamner 2020; Li et al. 2021; Lu et al. 2020; Miller et al. 2021; Park et al. 2020; Zhang et al. 2021).

In addition, the study at the meat packaging plants also supported airborne transmission, as well as corroborated laboratory studies that found environmental conditions such as low temperature and high humidity could lead to increased transmission (Dombrovsky et al. 2020; Günther et al. 2020). Environmental conditions impact surface and aerosol SARS-CoV-2 viability with warmer temperatures and sunlight increasing droplet evaporation and viral decay rates. Strategies to mitigate aerosol and surface transmission need to take into account the environmental conditions to yield the most successful results.

Sampling and Potential Recovery of SARS-CoV-2

Environmental sampling studies such as the ones performed in healthcare facilities provided evidence that higher viral loads were found in aerosols and surfaces when sampling was typically performed closer to the patient and early in the infection. In a review of healthcare studies, SARS-CoV-2 RNA was mostly found in less than 27% of the surface samples and less than 50% of the air samples, with only a few studies being able to successfully culture surface or air samples (Figure 1). While these studies provided insight into the areas that are the most heavily contaminated with SARS-CoV-2, their major limitation was the inability to specifically quantify the amount of viral load. The lack of studies finding viable virus, especially in the healthcare settings, where one would expect viable virus, could reflect inadequacy of current sampling methods. A negative RNA sample for SARS CoV-2 could indicate an absence of the virus or it also could indicate that the virus was present but was not found, as current methods are insufficiently sensitive. Similarly, a positive SARS CoV-2 RNA sample, indicates the presence of viral RNA fragments, but unless the sample is also cultured successfully, does not determine viability which is critical when discussing transmission. The limited number of studies in which there was successful culture indicates finding viable SARS-CoV-2 is likely more of a methodological problem than lack of viable virus. The lack of finding viable virus prevents occupational-based health decisions (like the types of PPE required) to be determined solely from sampling strategies.

Collection of viable SARS-CoV-2 in the air remains challenging in field studies. The inconsistent results from sampling across multiple healthcare studies are likely due to the variety of methods utilized (type, flow rate of aerosol sampling pump, and duration of collection), ventilation, percentage of fresh air, and air exchange rates, all of which illustrate the strengths and weaknesses within each study (Figure 2)(Dumont-Leblond et al. 2021; Dumont-Leblond et al. 2020). One of the many reasons for varied outcomes from aerosol sampling could be due to the potentially high air exchange rate in the hospital rooms, which varied from minimal air movement to 16 air changes per hour or was not recorded at all (Dumont-Leblond et al. 2020; Jin et al. 2021; Li et al. 2020; Tan et al. 2020). Additionally, sampling for SARS-CoV-2 was conducted in a variety of locations in hospitals including intensive care hospital patient rooms, general ward patient rooms, patient bathrooms, patient mobile toilet facilities, in hospital hallways, and during hospital PPE doffing (Chia et al. 2020; Guo et al. 2020; Jin et al. 2021; Kenarkoohi et al. 2020; Lei et al. 2020; Liu et al. 2021b; Pochtovyy et al. 2021; Razzini et al. 2020; Santarpia et al. 2020; Stern et al. 2021; Tan et al. 2020). The lack of culturable virus from air samples could be the result of potential damage done to the virus during high volume sampling or extended duration of aerosol sampling, as the virus can be lost at multiple stages of the collection process

(Brown et al. 2015; Dumont-Leblond et al. 2020; Jin et al. 2021; Pochtovyy et al. 2021). Only one study has evaluated performance of aerosol samplers, finding lower recovery of SARS-CoV-2 with midget impingers (Ratnesar-Shumate et al. 2021). The sampling collection method can have a large impact on maintaining viability of virus, and therefore yield inconclusive estimates of any infectious aerosol concentrations (Brown et al. 2015; Mainelis 2020).

As discussed previously, the proximity to an infectious person, their actions, and time relative to onset of illness, will impact the number of infectious particles that are being aerosolized or spread via bodily fluids, and therefore the number of viral particles that could be detected by sampling. Additional parameters affecting surface sampling include what type of surface was sampled, decontamination procedures in place where the surface was sampled, when the samples were collected relative to when disinfection measures were implemented, and when the surface was contaminated. A range of experimental conditions should be considered when evaluating the decay rate of SARS-CoV-2. The effects of 1) the selected viral suspension medium, including the similarity to respiratory secretions of an infected individual and whether preservative agents such as bovine serum albumin, fetal bovine serum, or both were integrated into the medium, 2) the droplet size of the suspension medium 3) the selected drying period duration prior to the onset of the experiment, and 4) the efficiency of virus recovery can all significantly influence the reported decay rate of SARS-COV-2. In addition, the differences in terminology for results between studies can make it difficult for comparisons, such as terms like decay rate and K-infectivity. When validation is impracticable, researchers should be aware of the limitations of the methods they select and provide detailed explanations of the methods so that the research can be appropriately assessed and compared with other studies.

Environmental parameters during air or surface sampling, such as temperature, relative humidity, and the presence or absence of UV radiation impacts the viability of the virus and, therefore, will also significantly impact the study results and limit the ability to compare results across studies. Not all elements of the methods and conditions are fully described within each study which makes cross-comparison of methods challenging and the experiments difficult to repeat.

Strategies for after-sampling methods for quantifying SARS-CoV-2 in air and on surfaces have varied across studies (Figure 3). While most sampling studies have utilized various air collection samplers or swabs on surfaces, methods for preserving samples during transport and storage, extracting SARS-CoV-2 RNA from sample medium, and quantitating viral nucleotides have differed from study to study. It is unclear whether these differences have significantly impacted the accuracy and precision of the results, but this lack of clarity has made it harder to compare results across studies. The impact of how samples are transported (what medium), the time period between collection and analysis (potential loss), and differences in the RNA extraction method between studies on method performance from an end-to-end (collection through analysis) perspective is poorly understood. Moreover, there is a high degree of variation in the number and combination of genes that have been targeted across studies, which may impact the sensitivity (more target genes lead to higher likelihood of detection) and comparability (due to differences in target genes combinations)

of study results. Determining if a sample has viable virus is performed with viral assays which are considerably more complex and difficult than bacterial or fungal assays (Cox et al. 2020). Lastly, many of these methods do not have a set limit of detection of the entire sampling procedure and the collection, including the initial capture method, or extraction efficiencies. Studies run controls with the RT-PCR analysis in order to determine the Ct value to establish sample positivity, but based on a review of hospital studies, the methods for RNA analysis and positivity Ct values varied from study to study (Birgand et al. 2020).

Without validating the method utilized including all the steps during the sampling and analysis processes, limited statements can be made regarding viral loads and infectivity. Public health decisions can only be made with reliable and verified methodologies, and therefore, until sampling can be performed with consistent and successful methodologies for SARS-CoV-2, sampling for viable virus cannot be a determining factor for workplace safety. This rapid review has demonstrated that there is a tremendous amount of work still to be done in regard to sampling for the quantification of SARS-CoV-2 in the environment.

Rapid review assessment

From June 2020 to September 2021, a comprehensive assessment of existing knowledge pertaining to SARS-CoV-2 transmission pathways was conducted biweekly using a rapid review framework. This rapid review quickly assessed very large amounts of information to get specific papers to subject matter experts. This approach allowed for real-time recommendations in the workplace to evolve as transmission pathways were better understood during the pandemic. This process enabled a small group of exposure assessment researchers to review most papers in their area of expertise as they became available. This group of exposure assessment researchers was also utilized to answer questions from workers, workplaces, and other government agencies using the most recent research. During the first summer of the pandemic, a comprehensive research review was used to write the NIOSH research agenda (NIOSH 2021) and also to fund many projects within NIOSH that addressed the research gaps.

The “knowns” of transmission changed rapidly during this period. Initially, contact and droplet transmission were deemed by the WHO as a critical exposure pathway, and as more scientific data became available, aerosol transmission was determined to be the primary pathway. As the pandemic progressed, researchers began to realize that infection and the onset of illness was important, as this varied from SARS-CoV-1, and articles began pointing out the timing of sampling in relation to onset of illness of SARS-CoV-2 patients. Similarly, sampling and environmental conditions such as ventilation, air exchange rates, and proximity of samplers to patients also became reported more frequently. Additionally, the understanding of pre-symptomatic and asymptomatic patients’ contribution to the role of transmission became evident (Wei et al. 2020a; Wei et al. 2020b). As stated earlier, keywords were utilized to narrow the focus of the SARS-CoV-2 research, the articles were screened, and if deemed significant, a summary was generated. Other critical topic areas, such as Engineering Controls or Personal Protective Equipment, had different search parameters and captured alternative articles that were not typically placed in more than one topic area, even though there could have been overlap in content. Therefore, subject

matter experts captured additional studies that were deemed highly significant outside of the framework of the rapid review search strategy. This approach yielded a large number of articles for qualitative review by Occupational Environment and Exposure Assessment subject matter experts. Perhaps a more concise transition of information, from keyword to selected summarized key articles, or a ranking system of articles based on expertise determined by number of the previously published peer-reviewed manuscripts in the field could have provided a more efficient real-time assessment.

Recommendations

Future SARS-CoV-2 sampling efforts would greatly benefit from the development of validated sampling methods in a laboratory prior to field sampling. The validation of sampling methods early in the pandemic would have allowed more accurate comparisons of results and appropriate interpretation of sampling findings. Without validated sampling and analysis methods, study findings can be difficult to interpret when addressing key questions, assessing exposure and transmission risk, and developing appropriate mitigation efforts.

While sampling methods to detect SARS-CoV-2 RNA and to isolate infectious SARS-CoV-2 are still developing, results are not currently suitable for the definitive determination of worker safety and transmission risk. Therefore, workers and managers must rely on best practices in order to minimize exposure. Workplace best practices, while not the focus of this literature review, are strategies that can be implemented to potentially reduce exposure including encouraging employees who are sick or have confirmed or suspected SARS-CoV-2 to stay home when possible. While this will reduce some transmission, asymptomatic and pre-symptomatic individuals can still spread the virus (Wei et al. 2020a; Wei et al. 2020b). Findings from the epidemiological, sampling, and experimental literature on SARS-CoV-2 have largely confirmed that many of the catch-all guidance measures for limiting airborne transmission of COVID-19, such as practicing social distancing and wearing masks, were and continue to be effective. Models that have estimated the impact of universal mask wearing have reported considerable reduction in transmission when mask-wearing compliance is over 50% (Tian et al. 2021). Other models have shown a clear reduction in transmission when maximum gathering and building capacity ordinances were enforced (Tian et al. 2021). Additional workplace strategies include supporting personal hygiene practices, improving workplace ventilation and filtration, utilizing outdoor areas as much as possible, reduced touching of common surfaces, and increased surface disinfection, among others.

Based on this literature review, our overarching findings include:

1. Sampling of workplaces will provide limited information for decision making until validated methods with known accuracy, precision, and limits of detection are developed and published, and consistent sampling strategies are established.
2. Aerosol transmission is an important pathway for SARS-CoV-2 transmission in occupational settings. Workplaces and workers should be trained to reduce this method of transmission, and administrative and engineering controls should be implemented to reduce person-to-person aerosol transmission as much as possible in the workplace.

3. Humidity extremes and low temperatures are conducive to survival of SARS-CoV-2 and create a greater risk of transmission; therefore, individuals working in places with those characteristics need to be aware and take precautions to reduce transmission, such as using high-quality face masks, and improving ventilation and filtration, if possible.

4. SARS-CoV-2 can exist in bodily fluids and is a workplace hazard for hospitals and anywhere else infectious individuals are found. It has a low chance of still being infective at wastewater processing facilities and is less of a risk for those workers.

5. A small portion of the population may be 'superspreaders' and produce more infective particles than others. This fact along with the fact that people can be asymptomatic or pre-symptomatic means that all people need to be treated as potential transmission sources.

6. Future research should focus on the modes of transmission (droplet, inhalational, direct and indirect contact) and determine the quantitative infectious dose of SARS-CoV-2 and its variants in varied occupational and community settings.

Limitations

While there are a vast number of articles included in this review, we have purposefully narrowed the research scope, and therefore have not included all SARS-CoV-2 research. Topic areas including Epidemiology, Personal Protective Equipment, and Engineering Controls were not included in this review unless determined by a subject matter expert to be critical. Therefore, by attempting to focus the research scope, all potentially relevant articles may not have been captured. Another limitation was the intention of a quick and efficient review over comprehensiveness, which could also contribute to relevant articles not being captured. In an effort to collect information with a consistent approach, subject matter experts reviewed articles bi-weekly to identify the studies that were most impactful, and qualitatively evaluated the caliber of the studies.

Conclusions

Rapid review in the midst of a pandemic was very useful for quickly understanding the transmission parameters of the virus and enabled us to comprehensively assess published and pre-published literature, respond to workplace questions, and evaluate our understanding as the science changed. Air and surface sampling has not been shown to be an effective strategy for determining worker exposure to SARS-CoV-2, as the recovery of viable virus in field sampling still needs to be developed and standardized. The development of validated methods for viable viral sampling and collection of viral RNA is necessary to provide exposure assessment tools for assessing worker risk. These methods along with the key parameters such as the environmental factors can help better explain transmission dynamics and the impact of mitigation strategies. While these strategies will vary from environment to environment, efforts should focus on mitigating risks associated with airborne transmission of the virus. The implementation of best practices in the workplace to help reduce exposures is currently the most effective strategy to reduce SARS-CoV-2 transmission.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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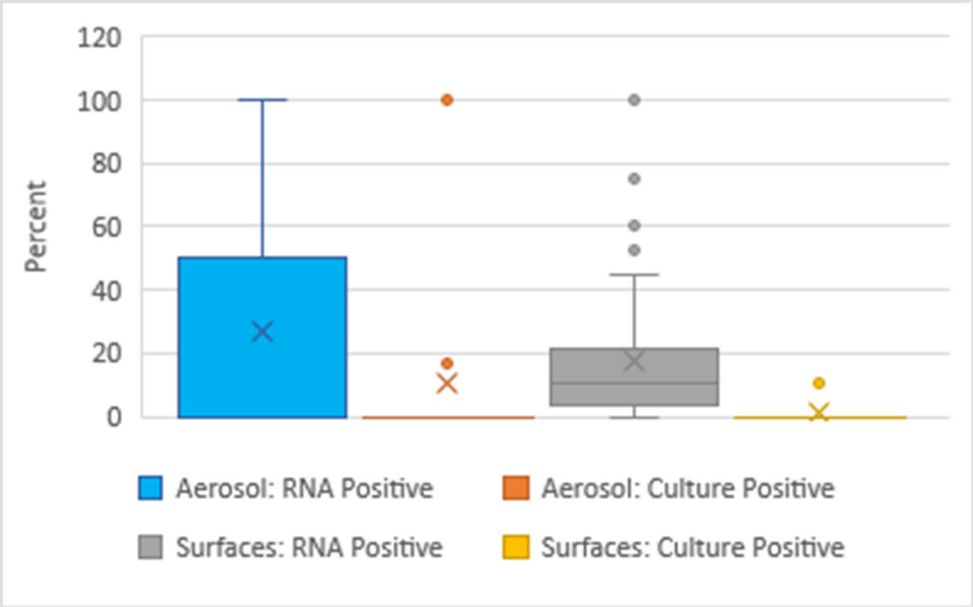

Figure 1.

Percentages of SARS-CoV-2 RNA and viability positivity rates across multiple studies for aerosol and surface samples. Aerosol data from Birgand (2020) RNA positive: 24 studies with 893 samples; Culture: 5 studies with 81 samples and surface data from Kampf (2021) RNA positive: 42 studies with 6914 samples; Culture: 8 studies with 886 samples. Boxplot represents the 25%, 50% and 75% quartiles. X represents mean.

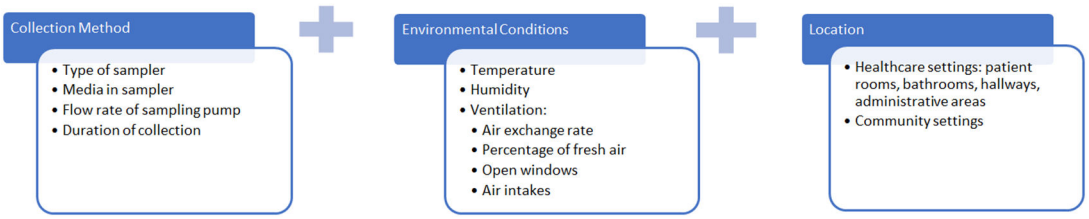

Figure 2.
Aerosol sampling variables that contribute to lack of precision between sampling efforts

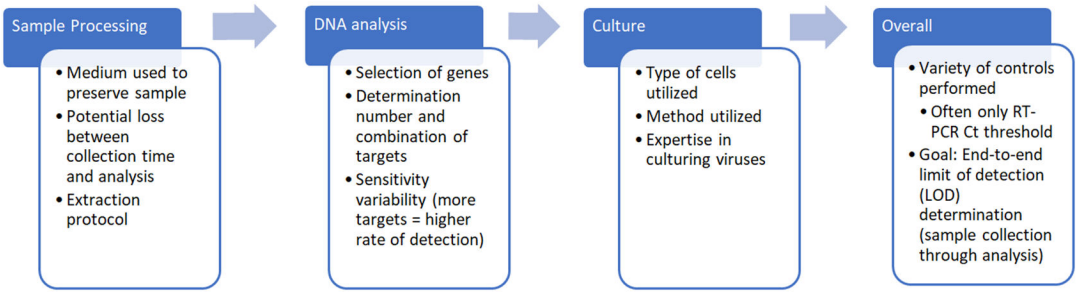

Figure 3.

After sample collection variability and issues that contribute to the lack of precision between sampling efforts.

Table 1.

Number of Articles by Topic Area and Exposure Matrix

Topic Area		Total	Bodily Fluids	Aerosols	Surfaces	Other*
Monitoring		117	17	59	68	25
	Attempted viral culture		7	24	25	8
	Isolated viable virus		6	12	11	3
	Evaluated presence of RNA**		7	55	65	21
Method Evaluation		13	0	5	9	2
Persistence of Infectivity/ Decay		80	15	12	61	13
Total***		197	33	78	131	40

*Captures studies monitoring for SARS-CoV-2 or evaluating monitoring methods in water (e.g. rivers or wastewater) or soil and a study assessing transfer of contaminants from an artificial human finger.

**The total reported includes literature reviews that did not experimentally assess viral culture or RNA presence. Studies may also have attempted viral culture.

***The number of studies may not sum to the total due to overlapping topic areas within a single study (e.g., a single publication monitoring surface contamination in a hospital as well as assessing persistence of infection on surfaces in a laboratory setting).

Table 2.

Number of Articles by Reference Type

Reference Type	Number of Publications
Literature Review	25
Epidemiology	52
Exposure Modeling	44
Articles included in other critical topic areas	8
Subject matter expert found research	22
Total *	151

* The number of studies may not sum to the total due to overlapping topic areas within a single study (e.g., a single publication monitoring surface contamination in a hospital as well as assessing persistence of infection on surfaces in a laboratory setting).