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ORIGINAL ARTICLE

Direct and quantitative capture of viable bacteriophages from experimentally contaminated indoor air: A model for the study of airborne vertebrate viruses including SARS-CoV-2

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Abstract

Aim: The air indoors has profound health implications as it can expose us to pathogens, allergens and particulates either directly or via contaminated surfaces. There is, therefore, an upsurge in marketing of air decontamination technologies, but with no proper validation of their claims. We addressed the gap through the construction and use of a versatile room-sized (25 m^3) chamber to study airborne pathogen survival and inactivation.

Methods and Results: Here, we report on the quantitative recovery and detection of an enveloped (Phi6) and a non-enveloped bacteriophage (MS2). The two phages, respectively, acted as surrogates for airborne human pathogenic enveloped (e.g., influenza, Ebola and coronavirus SARS-CoV-2) and non-enveloped (e.g., norovirus) viruses from indoor air deposited directly on the lawns of their respective host bacteria using a programmable slit-to-agar air sampler. Using this technique, two different devices based on HEPA filtration and UV light were tested for their ability to decontaminate indoor air. This safe, relatively simple and inexpensive procedure augments the use of phages as surrogates for the study of airborne human and animal pathogenic viruses.

Conclusions: This simple, safe and relatively inexpensive method of direct recovery and quantitative detection of viable airborne phage particles can greatly enhance their applicattion as surrogates for the study of vertebrate virus survival in indoor air and assessment of technologies for their decontamination.

Significance and Impact of the Study: The safe, economical and simple technique reported here can be applied widely to investigate the role of indoor air for virus survival and transmission and also to assess the potential of air decontaminating technologies.

KEYWORDS

aerobiology, aerosolization, air decontamination, airborne viruses, bacteriophages, coliphage MS2, indoor air, phage Phi6

INTRODUCTION

Although field-relevant testing of air decontamination technologies against airborne bacteria and fungi is feasible and practical (Sattar, Wright, et al., 2016a), assessments against airborne viruses in general remain challenging. Such studies need nebulization of relatively high-titred suspensions of viable/infectious airborne pathogens into a

typical room-sized chamber (http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0150-0025). Although most bacteria and fungi can be readily grown to the high titers needed (Verreault et al., 2008), that is often difficult with human and animal pathogenic viruses. Moreover, airborne bacteria and fungi can be recovered directly from the air on semi-solid culture media and their colonies counted; this is not possible with viruses because they are obligate parasites and need susceptible cell cultures or animals to quantify their infectivity (Sattar et al., 2015). Therefore, virus-containing aerosols must first be captured in a collecting fluid and the fluid assayed for viable/infectious viral units. The use of bacteriophages (phages) as surrogates for vertebrate viruses can be an alternative to overcome the diffculties, expense and constraints of working with vertebrate viruses (Dubuis et al., 2020; Turgeon et al., 2016; Verreault et al., 2008; Whitworth, 2020). Although making high-titered stock-cultures of phages is possible, a method for their direct capture from the air has not been available so far. As phages require bacterial hosts to infect and replicate in, they offer themselves as surrogates for vertebate viruses in this context. Here, we describe a method to directly recover an enveloped and a non-enveloped phage from the experimentlly contaminated air of an aerobiology chamber (Sattar, Wright, et al., 2016a) using a programmable slit-to-agar (STA) air sampler on nutrient agar plates with lawns of their respective bacterial hosts. This method was then applied to determine the rates of natural decays of the two types of phages and also to assess the activity of two commercial indoor air decontamination devices for phage removal/inactivation.

MATERIALS

The air decontamination devices

The air decontamination devices were installed and used according to the instructions from their respective manufacturers. Both devices were based on a combination of HEPA filtration and UV irradiation. For this study, the devices were tested separately by placing them in one corner of the aerobiology chamber and remotely operating them at their highest speed.

The air sampler

A programmable STA sampler (Particle Measuring Systems, Boulder, CO; https://pdf.directindustry.com/pdf/particle-measuring-systems/air-trace-environmental-slit-to-agar-sampler/28441-64025.html; accessed on July 18, 2021) was used to collect air samples from the aerobiology chamber at the rate of 28.3 L (1 ft.^{3})/min. The sampler was placed outside the chamber and the sampler's inlet was connected via a polyvinyl chloride (PVC) pipe to withdraw air from the aerobiology chamber. A fresh Petri plate (150 mm diameter) with a suitable nutrient agar was used to collect the phage via impaction from the air and the plates incubated for the development of plaque-forming units (PFUs). The air sample collection time varied from 2 to 10 min depending on the nature of the experiment. The plates for MS2 were incubated at 36 ± 1°C for 16 ± 2 h and those for Phi6 at 30 ± 1°C for the same length of time.

The Collison nebulizer

A six-jet Collison nebulizer (CH Tech., Westwood, NJ; www.inhalation.org) was used to separately generate the aerosols of the test phage for 10 min. Air from a compressed air cylinder at ~172 kPa (25 psi) was used to operate the nebulizer.

Phages tested and their culture and quantitation

Phage MS2 (ATCC 15597-B1) was grown in its bacterial host *Escherichia coli* (ATCC 15597). This phage is a relatively small (about 27 nm), non-enveloped virus that is frequently used as a surrogate for human pathogenic viruses (Turgeon et al., 2016). It has a linear genome of single-stranded RNA. It belongs to the family *Leviviridae*. This virus was a gift from the Bureau of Microbial Hazards, Health Canada, Ottawa, ON, Canada.

Cystovirus Phi6 (ATCC 21781-B1), an enveloped phage with a spherical virion of ~85 nm in diameter, was used with its host *Pseudomonas syringae* subsp. *syringae* (ATCC 19310). The virion has a double-capsid structure with a three-segmented dsRNA genome. It is a lytic bacteriophage of Gram-negative plant pathogenic bacteria (International Committee on the Taxonomy of Viruses 2020). It belongs to the family *Cystoviridae*. Seed cultures of the virus and its host cells were purchased from l'institut Felix d'Herelle, Université Laval, QC, Canada.

A frozen stock culture of the host bacteria was thawed and streaked onto Luria Bertani (LB) agar and incubated to isolate an individual colony for sub-culturing into LB broth to be used in the 150 mm LB bilayer agar plate host lawn preparation for the STA as well as preparation of the bacteriophage stock culture. The hard (bottom) agar was prepared by pouring 60 ml of 1.5% LB agar into 150-mm Petri plates containing 16 ml of 1% triphenyl tetrazolium chloride (TTC) per/L. The agar concentration in soft (top)

agar for preparation of bacteriophage stock and STA sampling were 3% and 8%, respectively.

To prepare a bilayer agar plate, the top agar was autoclaved and poured into 50-mL tubes (25 ml in each). The 50-mL tubes were placed in a water bath at 48ºC. After the agar had equilibrated to the water bath temperature, and the concentration of the bacterial host reached 0.65–0.75 OD600, 1.5 ml of host bacterial culture with 100 μl of 1 M $CaCl_2$ and 1 ml of 1% TTC were added to the top agar tube, mixed by gentle inverting 10–15 times and poured on top of a 150 mm Petri plate containing bottom agar. For preparation of the phage stock, 100 μl of phage suspension was also added to the top agar tube before mixing. The bilayer agar plates were left under a Biological Safety Cabinet (BSC) for at least 15 min before using them for sampling or moving to the incubator for making phage stock. The incubation conditions for each type of host bacterium are summarized in Table 1.

At the end of the required incubation period, the phages were separately harvested by collecting the top agar layer from the bilayer Petri plate with a spreader into 50-ml conical centrifuge tubes. A calcium saline solution ~1 ml was used to help liquefy the soft agar. The 50-ml tubes were vortexed and then centrifuged at 10,000 × g for 20 min. After centrifugation, the supernatant was poured into a 60 cc syringe and passed through a 0.2 μm pore diameter membrane filter. The supernatants were pooled and aliquoted into 1 ml volumes then stored at −80°C. Assays for PFU were performed using the bilayer method (Cornax et al., 1990).

Aerobiology chamber

The aerobiology chamber used for this study was a 25 m^3 enclosure; the chamber has been described in detail previously (Sattar, Wright, et al., 2016a). It was constructed to conform to the U.S. Environmental Protection Agency's guideline for facilities to assess microbial decontamination of indoor air (http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0150-0025).

TABLE 2 Levels of airborne phage contamination achieved in the aerobiology chamber after 10 minutes of nebulization

	Log_{10} PFU/m^3 of chamber air		
Test	**Control**	**Test 1**	**Test 2**
MS2	4.82	4.82	4.80
Phi6	4.00	4.36	4.55

The chamber was fitted with a PVC pipe connected to a six-jet Collison nebulizer outside the chamber to allow for microbial contamination of the air. Another PVC pipe, connected to an externally placed STA sampler, directly impacted the sampled air onto the surface of the semi-solid microbial recovery medium. A muffin fan 7.5 cm diameter (3 inch) was positioned under the nebulizer inlet pipe and actuated from outside the room during nebulization to uniformly distribute the aerosol particles suspended in the air. Between experiments, the chamber was purged with HEPA-filtered air to remove any residual microbial contamination. The relative humidity (RH)/air temperature inside the chamber were monitored and recorded during each experiment using a wireless sensor/data logger system (Dickson).

RESULTS

All tests were conducted with the ambient RH between 50 ± 5% and the air temperature at 22 ± 2ºC. Table 2 shows the levels of viable airborne phages achieved in the chamber after 10 min of nebulization. The data for the performance of the two devices is given in Table 3.

Figure 1 shows culture plates with the plaques of the two phages directly captured from experimentally contaminated air with an air sampling time of two minutes. The plaques were evenly distributed over the plates and readily countable after an incubation of 18 ± 2 h.

As shown in Figure 2, three separate tests were conducted with each one of the two phages to determine the rates of their natural (biological) decay. The decay rate of

TABLE 1 Growth conditions for the culture of the bacterial hosts and plauqe assay of MS2 and Phi6 phages

Factor	MS2	Phi6
Host bacterium	*E. coli*	*P. syringae*
Liquid culture medium for gowing the host bacterium	LB broth to reach OD 0.65–0.75 on a shaker at 225 rpm	LB broth to reach OD 0.65–0.75 on a shaker at 225 rpm
Semi-solid culture medium for plaques	LB bilayer agar	LB bilayer agar
Incubation temperature to grow the host cells	36 ± 1°C	30 ± 1°C
Incubation time for plaque development	18 ± 2 h	18 ± 2 h

TABLE 3 Efficacy of two different air decontamination devices against MS2 and Phi6 phages

	MS2		Phi6	
	Time to demonstrate a 3 $\log_{10}$ reduction in viability	**Time to reduce the viability of the phage to an undetectable level**	**Time Demonstrating a 3-$\log_{10}$ reduction**	**Time to reduce the viability of the phage to an undetectable level**
Device #1	7.4 min	4.16 $\log_{10}$, 15 min	11 min	3.69 $\log_{10}$, 15 min
Device #2	62·5 min	4.09 $\log_{10}$, 85 min	Could not demonstrate 3-$\log_{10}$ reduction	1.57 $\log_{10}$, 86 min

MS2 was found to be 0.006 ± 0.001/min while that of Phi6 was 0.040 ± 0.002/min.

Two different commercial devices for indoor air decontamination were tested against the two phages. The test protocol was as described previously (Sattar, Wright, et al., 2016a), except with the use of a bilayer of LB agar plates containing the host bacteria instead of Trypticase soy agar plates.

Figure 3 shows the $\log_{10}$ reductions by the two devices against MS2. The performance of the two devices are summarized in Table 3. Device #1 demonstrated a 3 $\log_{10}$ reduction in the viability of MS2 in 7.4 min and 11 min, while Device #2 needed 62.5 min to reach a 3 $\log_{10}$ reduction.

Figure 4 shows the activity of the two devices against phage Phi6 and its maximum 3 $\log_{10}$ decontamination against Phi6 was 1.57 after 86 min.

DISCUSSION

The two phages were selected to represent airborne enveloped and non-enveloped vertebrate viruses (Sattar, Zargar, et al., 2016b). Since making high titer stock-cultures of bacteria and phages is relatively easy and practical, they can be used for field-relevant studies of air decontamination. The use of phages also does not require elaborate biosafety precautions due to their non-pathogenicity to other life-forms. The ease and speed of culture of their respective bacterial hosts using relatively simple media also makes the test systems economical while giving a rapid (within 24 h) turn-around of the results.

Although vital staining of the STA plates was not essential, it did enhance the ease with which the plaques could be observed and counted (Figure 1).

Although all-glass impingers and sieve samplers (Sattar & Ijaz, 1987; Verreault et al., 2008) can also be used to recover airborne microbes, only the use of a programmable STA air sampler would allow for a better and direct visualization of the test microbe's rate of physical/biological decay in the air as well as an event-related profile of microbial contamination/decontamination without and with the air decontaminating devices in operation.

In this study, the STA used was purchased from Particle Measuring Systems (Boulder, CO). Since that device is no longer made, we have found a suitable replacement for it made by Pinpoint Scientific Company (https://www.pinpointscientific.com/technology-and-specification) located in the U.K.

The test systems reported here also allowed us to assess the activity of commercial indoor air decontamination devices with considerable ease, economy and safety, thus laying the foundation for a standardized test protocol for

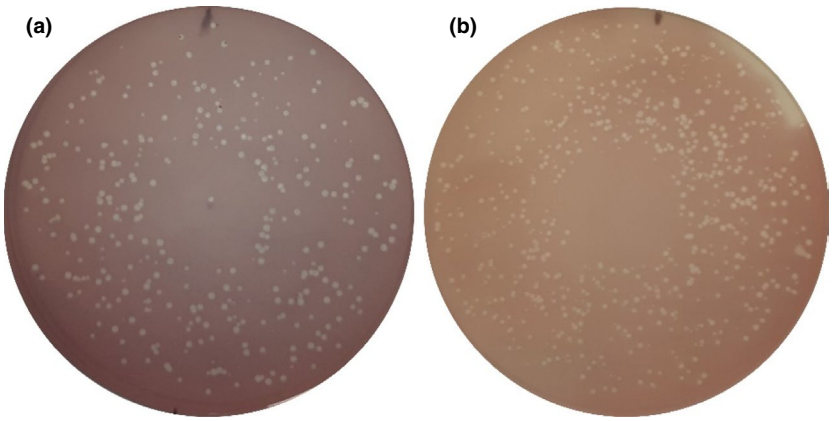

FIGURE 1 Petri plates with the plaques of the two phages as captured directly from experimentally contaminated indoor air. (a) Plaques of MS2 on a lawn of *E. coli*; (b) Plaques of Phi6 on a lawn of *P. syringae*. (b) The plates with the air samples were incubated for 24 ± 2 h and stained with the vital dye TTC to better visualize the plaques

technology assessments and also pre-market label claims of such technologies.

In this study, no soil load was added to the phage suspensions to be aerosolized (Springthorpe & Sattar, 2007). Therefore, further testing may be needed to determine the influence of an added soil load on the rate of biological decay of the phages as well as the decontamination ability of the tested devices on experimentally contaminated air.

The shortest air sampling time of the STA used was two minutes and the longest five hours during which the agar plate inside it completed one full rotation. If the concentration of viable airborne phages was high, a sampling time of even two minutes may result in too high a number of plaques to allow for proper counting. In such cases, the starting concentration of the test phage in the air required reduction to enable a more accurate PFU count. The longest duration of sampling time did not damage the viability of the host cell lawn due to drying. We also did not encounter any extraneous (Andersen, 1958) airborne bacterial or fungal contamination on the STA plates as the testing was conducted in an aerobiology chamber where in-coming and out-going air was HEPA-filtered.

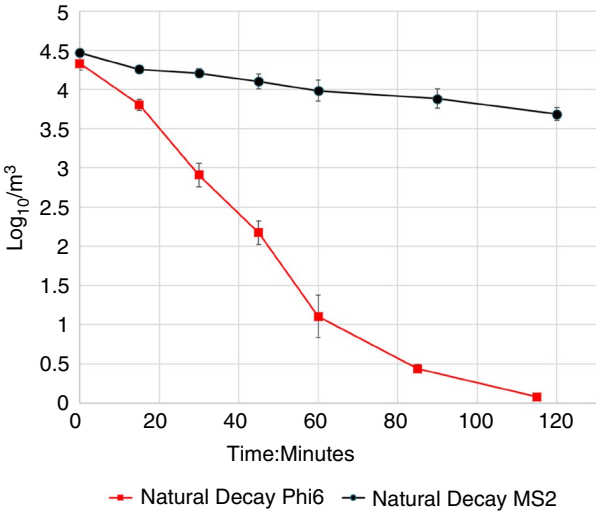

FIGURE 2 Natural decay profiles of phages MS2 and Phi6 arosolized into indoor air. The phages were separately suspended and aerosolized using a Collison nebulizer into an aerobiology chamber. The aerosols were held at 23 ± 3ºC at an RH of 50 ± 10ºC. Samples of the air from the chamber were collected over two hours using an slit-to-agar sampler on agar plates prepared with a suspension of the respective host bacteria. The plates were incubated for 24 ± 2 h, stained with the vital dye TTC to enhace the visibility of the plaques. The plaques were counted to determine the rates of natural decay

To our knowledge, the only published study on the direct capture of an airborne phage is that of Jensen (Jensen, 1967). In that study, an Andersen sieve sampler was used to collect a tailed phage (T7) on the lawns of its *E. coli* host. The Andersen air sampler is well recognized for its ability to separate airborne particles into their respirable size range (Andersen, 1958) but unlike an STA, it is incapable of generating an event-related profile of viable airborne particles.

The protocol described is flexible enough for work with other phages and their respective bacterial hosts. In addition to use of phages described here as surrogates for enveloped and non-enveloped vertebrate viruses for asessment of air decotamination technologies (Dubuis et al., 2020; Duchaine, 2016; Turgeon et al., 2016; Whitworth, 2020), specific phage-bacterial host combination may also be used to monitor air contamination in biotechnology, brewing and dairy operations (Samson & Moineau, 2013).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

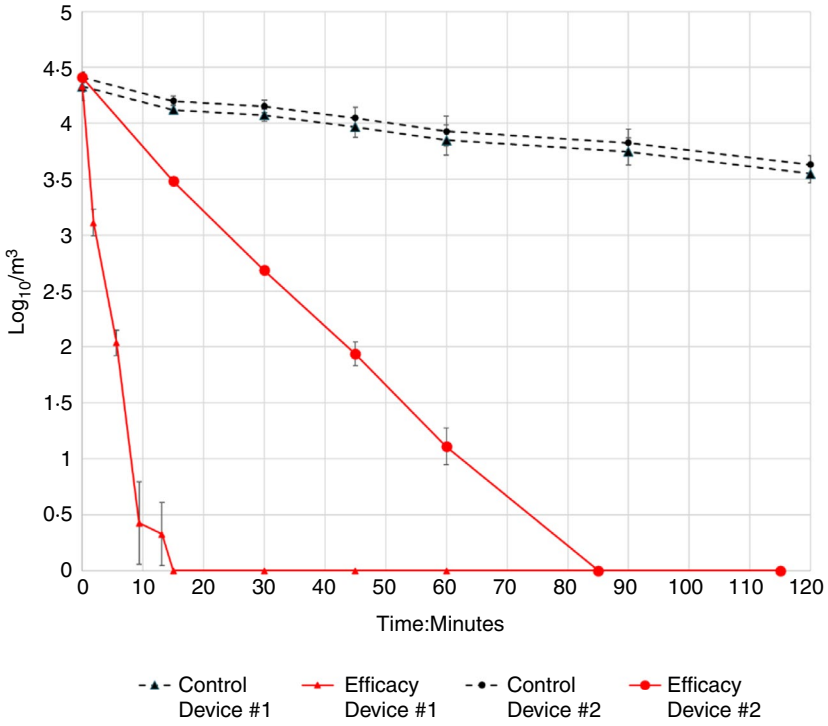

FIGURE 3 Activity of two different indoor air decontamination devices against phage MS2. The indoor air decontamination devices were tested separately in the aerobiology chamber and operated at their high-speed setting. Samples of experimentally contaminated air from the chamber were collected over 2 h using an slit-to-agar air sampler. The sampler contained agar plates prepared with the lawn of the *E. coli* host. The plates were incubated for 24 ± 2 h, stained with the vital dye TTC and plaques on them counted

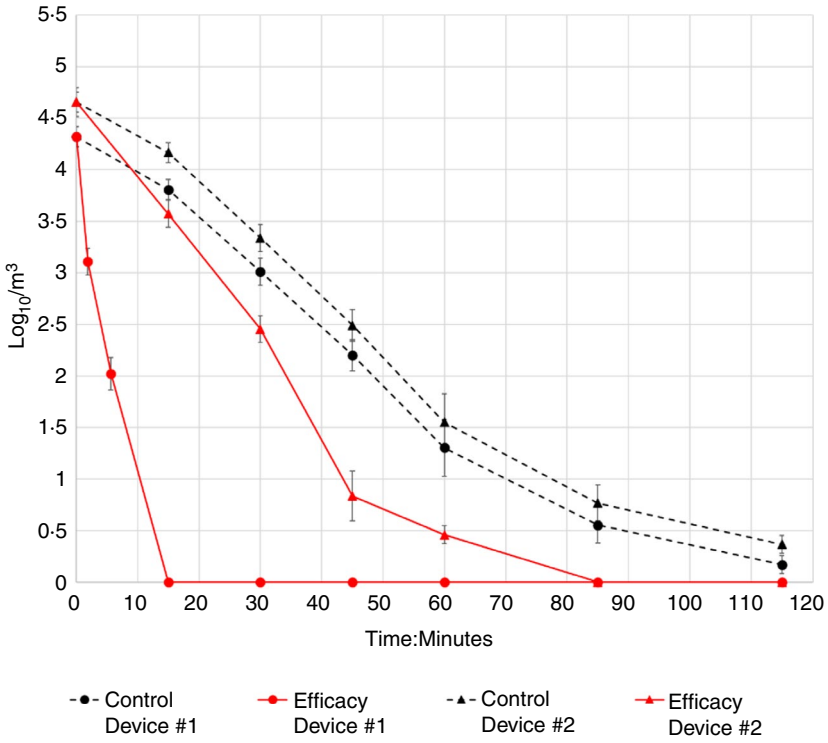

FIGURE 4 Activity of two indoor air decontamination devices against phage Phi6

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REFERENCES

Andersen, A.A. (1958). New sampler for the collection, sizing, and enumeration of viable airborne particles. *Journal of Bacteriology*, 76(5):471–484.

Cornax, R., Moriñigo, M.A., Paez, I.G., Muñoz, M.A. & Borrego, J.J. (1990) Application of direct plaque assay for detection and enumeration of bacteriophages of Bacteroides fragilis from contaminated-water samples. *Applied and Environmental Microbiology*, 56(10), 3170–3173.

Dubuis, M.E., Dumont-Leblond, N., Laliberté, C., Veillette, M., Turgeon, N., Jean, J. et al. (2020) Ozone efficacy for the control of airborne viruses: bacteriophage and norovirus models. *PLoS One*, 15(4), e0231164.

Duchaine, C. (2016) Assessing microbial decontamination of indoor air with particular focus on human pathogenic viruses. *American journal of infection control*, 44(9 Suppl), S121–S126. https://doi.org/10.1016/j.ajic.2016.06.009.

Jensen, M.M. (1967) Bacteriophage aerosol challenge of installed air contamination control systems. *Applied Microbiology*, 15(6), 1447–1449.

Samson, J.E. & Moineau, S. (2013) Bacteriophages in food fermentations: new frontiers in a continuous arms race. *Annual Review of Food Science and Technology*, 4, 347–368.

Sattar, S.A., Bradley, C., Kibbee, R., Wesgate, R., Wilkinson, M.A., Sharpe, T. et al. (2015). Disinfectant wipes are appropriate to control microbial bioburden from surfaces: use of a new ASTM standard test protocol to demonstrate efficacy. *Journal of Hospital Infection*, 91(4):319–325.

Sattar, S.A., Ijaz, M.K. (1987) Spread of viral infections by aerosols. *Critical Reviews in Environmental Control*, 17(2), 89–131.

Sattar, S.A., Wright, K.E., Zargar, B., Rubino, J.R. & Ijaz, M.K. (2016a) Airborne infectious agents and other pollutants in automobiles for domestic use: potential health impacts and approaches to risk mitigation. *Journal of Environmental and Public Health*, 2016, 1–12, https://doi.org/10.1155/2016/1548326.

Sattar, S.A., Zargar, B., Wright, K.E., Rubino, J.R., Ijaz, M.K. & Kibbee, R.J. (2016b) Decontamination of indoor air to reduce the risk of airborne infections: Studies on survival and inactivation of airborne pathogens using an aerobiology chamber. *American Journal of Infection Control*, 14(10), E177–E182.

Springthorpe, V.S. & Sattar, S.A. (2007) Application of a quantitative carrier test to evaluate microbicides against mycobacteria. *Journal of AOAC International*, 90(3), 817–824.

Turgeon, N., Michel, K., Ha, T.L., Robine, E., Moineau, S. & Duchaine, C. (2016) Resistance of aerosolized bacterial viruses to four germicidal products". *PLoS One*, 11(12), e0168815.

Verreault, D., Moineau, S. & Duchaine, C. (2008) Methods for sampling of airborne viruses. *Microbiology and Molecular Biology Reviews*, 72(3), 413–444.

Whitworth, J. (2020) COVID-19: a fast evolving pandemic. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 114(4), 241–248.

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