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State of the Science Review

Assessing microbial decontamination of indoor air with particular focus on human pathogenic viruses

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Although the microbial world is rich in diversity, only a small portion of microbes represent a risk to human and animal health. However, the socioeconomic impact of such harmful microbes is enormous and represents an important worldwide challenge in public and occupational health and in veterinary medicine. Among the vehicles for microbial spread, indoor air is perhaps the least understood, likely because of a general lack of standardized protocols to study the survival and removal or inactivation of airborne microbes. This is a brief review of airborne transmission of infectious agents, along with an assessment of available technologies for the decontamination of indoor air, with particular reference to human pathogenic viruses.

According to Roy and Milton, certain types of pathogens are obligated to spread by air only; pulmonary tuberculosis is a good example of this. Others may do so preferentially (eg, measles, varicella), and still others may be opportunistic with regard to their airborne spread (eg, smallpox, influenza, noroviruses). There are still others that may be carried by air to multiply in their host. For example, methicillin-resistant Staphylococcus aureus (MRSA) nasal carriage has been linked to exposure to contaminated air.

For some airborne infectious agents, the respiratory system may not be the ultimate target. For example, epidemiologic evidence suggests that airborne particles of human norovirus, a major cause of acute gastroenteritis, may first be retained in the tonsillar region, with subsequent translocation to the gastrointestinal tract. Recently, molecular analysis of air found evidence of norovirus in several areas of health care facilities. The pandemic potential of human influenza viruses is related to their ability to spread by air. In light of this evidence, safe and effective decontamination of indoor air would be an important adjunct to infection prevention and control.

For most viral infections of humans, epidemiologic profiles correspond to direct-contact transmission through coughing, sneezing, or speaking-related emissions of pathogen-containing droplets and subsequent contact with the mouth or nose of a susceptible host. Droplets emitted by an infected person vary in size between 0.3 and 2.0 μm. Although the general size range of pathogen-laden droplet nuclei is 0.5-5.0 μm, it is hypothesized that the microbe itself has little influence in this regard. The size of such particles is driven mainly by their solute content.

The water content of air will influence the rate at which droplets will evaporate to become droplet nuclei (Fig 1). Droplet nuclei are preferentially formed at low relative humidity (RH), whereas high RH may favor maintenance and settling of droplets.
Influenza viruses were measured in the air of hospital emergency rooms in a National Institute for Occupational Safety and Health study. Over 50% of the detected viruses were found in the <5 μm fraction, suggesting their presence in airborne droplet nuclei. Similar findings were obtained with other respiratory viruses: cytomegalovirus, respiratory syncytial virus, rhinovirus, and the coronavirus responsible for the severe acute respiratory syndrome. Influenza viruses were measured in the air of hospital emergency rooms in a National Institute for Occupational Safety and Health study. Over 50% of the detected viruses were found in the <5 μm fraction, suggesting their presence in airborne droplet nuclei. Similar findings were obtained with other respiratory viruses: cytomegalovirus, respiratory syncytial virus, rhinovirus, and the coronavirus responsible for the severe acute respiratory syndrome. Exclusion of potentially harmful microbes and other factors simultaneously, making risk assessment a major challenge. For instance, individuals with preexisting respiratory allergies may react to an inhaled pathogen differently than individuals without respiratory allergies. Chronic smoking is also well known as a predisposing factor to respiratory pathogens.

In spite of the availability of a variety of methods for collecting microbes from indoor air, efficient recovery and detection and quantitation of viable pathogens in field samples of air remain difficult. The generally low levels of airborne pathogens require the collection of hundreds of liters of air, and such a process can be efficiently sampled and detected. Further, with a simple smoke test, it is possible to detect the less ventilated zones where pathogenic agents have higher odds of being concentrated. Inclusion of potentially harmful microbes and other factors simultaneously, making risk assessment a major challenge. For instance, individuals with preexisting respiratory allergies may react to an inhaled pathogen differently than individuals without respiratory allergies. Chronic smoking is also well known as a predisposing factor to respiratory pathogens.

EXPOSURE ASSESSMENT: FROM SAMPLING TO ANALYSIS

Indoor air often contains a varied and variable blend of microbes, along with a cocktail of chemicals, allergens, and other particulates. Inhalation of such air may expose an individual to a combination of potentially harmful microbes and other factors simultaneously, making risk assessment a major challenge. For instance, individuals with preexisting respiratory allergies may react to an inhaled pathogen differently than individuals without respiratory allergies. Chronic smoking is also well known as a predisposing factor to respiratory pathogens.

In spite of the availability of a variety of methods for collecting microbes from indoor air, efficient recovery and detection and quantitation of viable pathogens in field samples of air remain difficult. The generally low levels of airborne pathogens require the collection of hundreds of liters of air, and such a process can be quite damaging to the viability of many types of pathogens, leading to an underestimation of their concentration. Often, the pathogen recovered may not grow in the laboratory. In addition, molecular techniques have higher odds of being concentrated. Inclusion of potentially harmful microbes and other factors simultaneously, making risk assessment a major challenge. For instance, individuals with preexisting respiratory allergies may react to an inhaled pathogen differently than individuals without respiratory allergies. Chronic smoking is also well known as a predisposing factor to respiratory pathogens.

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Among the major knowledge gaps in the aerobiology of human pathogens is the lack of understanding of size distribution of airborne particles carrying viable infectious agents. Such knowledge (granulometry) will be crucial to the design, assessment, and deployment of indoor air decontamination technologies.

PHAGES AS MODELS FOR AIRBORNE VIRUSES

Phages are already used as models in several areas of research and field investigations. For example, in the pharmaceutic and food industries, the U.S. Food and Drug Administration recommends their use to test the effectiveness of filtration devices. They are also used as surrogates for enteric viruses in studies of wastewater treatment. However, their potential as surrogates in the study of aerobiology of human pathogenic viruses remains underexplored, despite their common structural similarities with eukaryotic viruses. For example, phages can be enveloped or nonenveloped and can possess single- or double-stranded RNA or DNA genomes, which may be segmented, linear, or circular. The phage capsids also are of a variety of sizes and shapes reflective of human pathogenic viruses. Our ability to culture and assay phages inexpensively and without the need for biosafety precautions also adds to their attraction as surrogates.

Recently, phage models have been developed and compared for appropriateness in simulating eukaryotic viruses in bioaerosols. The resistance of various phages to environmental stresses (RH, ultraviolet [UV], temperature, and aerosol duration) was studied, and it was shown that the response to stresses varied between the various models. Phage MS2 has been the most broadly used surrogate in aerosol studies and is used mostly in biodetection to predict the fate and transport of biothreat agents. Table 1 presents the phage models used and validated.

Our laboratory has used phages to predict the most probable areas in a mechanically ventilated building where airborne viruses could be efficiently sampled and detected. Further, with a simple smoke test, it is possible to detect the less ventilated zones where pathogenic agents have higher odds of being concentrated.

IN VITRO ASSESSMENT OF MICROBICIDAL AGENTS AND PROCESSES FOR INDOOR AIR DECONTAMINATION

Pathogenic agents may remain suspended in indoor air even in the absence of the infected person who is emitting them. Hence, air decontamination should be implemented in situations such as room cleaning after the release of an infected patient or after a vomiting episode in a classroom. In the literature, most of the procedures developed to decontaminate air in occupied spaces were not validated in vitro with multiple model microorganisms or size-distributed microbial aerosols. Although it would be highly desirable to assess any indoor air decontamination technology against all major types of airborne microbial threats before its adoption, time and cost constraints and the unavailability of suitable test protocols essentially preclude such an approach. Furthermore, the in-field efficiency of a given technology is also subject to numerous site-specific variables. This reinforces the need for well-designed experimental settings and robust test protocols and the selection of suitable surrogates for airborne pathogens to evaluate potential means of indoor air decontamination as thoroughly as possible. It should also be noted here that experimental aerosolization of infectious agents may increase the risk of biohazards in general, therefore requiring the need for proper staff training, the availability of proper personal protective equipment, and the institution of rigorous safety procedures.

Environmentally controlled aerosol-aging chambers are designed to simulate environmental stresses that are imposed on microbial aerosols in order to understand the role of environmental parameters, such as temperature, UV, and RH, on the fate of airborne infectious agents. Aerosols can remain airborne for prolonged periods in rotating chambers because these particles remain suspended in a rotating mass of air. The gravitational forces exerted on the particles are countered by centrifugal forces created by the rotation of the drum. The effects on various viral and bacterial aerosols held at different levels of air temperature, RH, hydrogen peroxide vapor, UV radiation, ozone, and other physical and chemical agents can be studied using the rotating drum (Caroline Duchaine, 2016). Figure 2 shows a picture of a rotating drum with the desiccants and the control panel.

AIR DECONTAMINATION FOR CONTROL OF INFECTIOUS AGENTS

Natural ventilation is the most important means of air decontamination, but it is not often applicable because of building design, climate, security, or pest control. Mechanical ventilation is more
expensive but can be effective if well designed. Portable air-cleaning systems that incorporate filtration, microbicidal UV irradiation, or other disinfection technologies can be installed in occupied spaces, but unless very well designed, portable equipment does not filter large volumes of air, and short circuiting can occur.\(^23\) The airborne environment is intrinsically hostile and stressful to infectious agents because of desiccation, radiation, and osmotic pressure, but little is known about the actual mechanisms of their inactivation. No universal approach is available, and decontamination techniques must be adapted to the target agent in place, given the agent’s relative resistance and robustness. However, some simple approaches have been developed for reducing the concentration of airborne infectious agents. The following are some examples.

### Temperature and RH

Air temperature plays a role in lipid stability, and decreases in temperature tend to stabilize the lipid layers of, for example, enveloped viruses, such as influenza viruses.\(^23\) \(^{24}\) RH also affects the viability of viruses.\(^24\) As a function of the genetic material (RNA or DNA) and the presence or absence of a lipid envelope, virus resistance to RH or temperature can vary, but it can be generalized that enveloped viruses (eg, influenza, coronavirus, respiratory syncytial viruses, parainfluenza viruses) are more stable under conditions of low RH and low temperature.\(^{35,36}\) The influence of RH and air temperature on phages used as surrogates for human pathogenic viruses was assessed in the laboratory using the rotating aerosol chamber.\(^28\) Results suggest that viruses behave differently and that no standard or generalized conclusion can be drawn regarding the virucidal effects of temperature and RH. However, modulation of RH and temperature in buildings or care facilities could be a promising approach to controlling the spread of some specific types of viruses in indoor air. Notably, some viruses, such as noroviruses, are very resistant to aerosolization stresses and environmental stresses that do not seem to affect infectivity.\(^7\)

### Ozone

Ozone is a normal atmospheric constituent produced naturally by the effect of UV rays on oxygen. At ground level, ambient concentrations normally range between 0.005 and 0.05 ppm.\(^37\) Ozone can be generated from ambient-air oxygen using UV light, laser, high voltages, electrostatic discharge, or chemical reaction.\(^38\) This unstable and highly oxidative gas is often used for disinfection of wastewater and potable water. Air decontamination could be a potentially useful application for high-scale use of ozone in building air exchange and ventilation systems. However, the effective use of

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Table 1

| Phages  | MS2          | Phi6         | PR772        | PhiX174       |
|---------|--------------|--------------|--------------|---------------|
| Family  | Leviviridae  | Cystoviridae | Tectiviridae | Microviridae  |
| Capsid  | One structural protein | Icosahedral | 27 nm       | Icosahedral | 53 nm       | Icosahedral | 23 nm       |
| Envelope| no           | yes          | no           | no            |
| Genome  | Single stranded | Linear RNA  | Non segmented | 3569 nucleotides | Double stranded | Linear RNA | 3 segments | 13 386 base pairs | Double stranded | Linear DNA | Non segmented | 14 402 base pairs | Single stranded | Circular DNA | Non segmented | 5388 nucleotides |
| Host    | E. coli      | Pseudomonas syringae | E. coli      | E. coli       |
| Incubation temperature | 37°C | 25°C | 37°C | 37°C |

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Fig 2. Rotating chamber for the study of aging bioaerosols and effectiveness of air decontamination approaches.
ozone in these systems requires concentrations > 5 ppm, concentrations at which ozone represents a risk for occupants. Ozone decontamination efficiency at low concentrations in ambient air has yet to be validated.

Ultraviolet

In hospitals, UV disinfection of upper-room air is being used as a cost-effective means of reducing the risk of airborne spread of infections.\(^\text{23}\) Inactivation of microbial agents in indoor air has been addressed using photocatalysis as a function of the optimizing power of ultraviolet radiations (A (UVA)–irradiated semiconductors.\(^\text{40,41}\) UV treatment involves the use of a photoreactor, some of them being commercially available, in which air is drawn through and particles impact on the photocatalytic surface. The efficiency of air decontamination varies with aerosol size because smaller aerosols are less likely to contact the decontamination surface.\(^\text{40}\) UV lamps have traditionally been applied to reduce aerosol transmission of Mycobacterium tuberculosis, and the potential for UV to kill a variety of vegetative cells in air is not without merit. UV irradiation has shown efficacy against certain fungal spores, such as those of Aspergillus spp, and for removal or inactivation of microbial aerosols at significant rates; however, this technology was not at the time of the study applied on a routine basis during outbreaks.\(^\text{42}\) Use of a combination of systems and technologies is worth studying; as an example, a 1% concentration of hydrogen peroxide can increase UV’s lethality 2,000-fold.\(^\text{43}\)

Hydrogen peroxide and other microbicides

Nebulization of microbicides in occupied spaces can be performed when the agent is not toxic for humans or corrosive for materials. Hydrogen peroxide has a low toxicity and is safe for most materials. Nebulized hydrogen peroxide delivered in the form of dry mist or vapor has shown efficacy for the reduction of health care–associated infections.\(^\text{44}\) In hospitals, highly resistant pathogenic microbes, such as Clostridium difficile spores, are known to be present in the air.\(^\text{45,46}\) In hospital rooms, nebulized hydrogen peroxide has been shown to reduce surface contamination by both C. difficile spores and MRSA, in fact contributing to eradication of persistent environmental contamination with MRSA.

Studies have also shown the virucidal effects of natural compounds such as essential oils (eg, eucalyptus oil, tea tree oil).\(^\text{47,48}\) These studies demonstrated the complete loss of viability of influenza virus, and nonenveloped phage M13, when exposed to aerosolized oils for >30 seconds; however, concentrations were harmful. Other materials, such as eugenol, a natural oil, and several commercially available air sanitizers were tested against aerosolized viruses (phage surrogates) and shown to have an efficacy that varied with RH and the phage type (ie, enveloped or nonenveloped, RNA or DNA) (Caroline Duchaine, 2016).

Electrostatic precipitation

An electrostatic precipitator (ESP) is a device that removes airborne particles by charging the particles with an electric field and then attracting them to charged collector plates. In laboratory settings, an ESP has demonstrated its air filtration effectiveness over a wide range of particle sizes,\(^\text{50,51}\) and efficacy of bacterial and fungal aerosol capture has been studied as well.\(^\text{51}\) An ESP has been used for enhancing indoor air quality in industrial settings and in homes and public buildings. It has also been used, but without success, in bedrooms during the night to improve peak expiratory flow rates of asthmatic children.\(^\text{52}\)

Filtration

Mechanical, microbicidal, and electrically charged fibrous filters are commercially available and used in heating, ventilation, and air conditioning systems. Usually, because these filters are not washable, an upstream prefilter is recommended for eliminating coarse particles and extending the life span of the filter. Antimicrobial and electrically charged fibrous filters are composed, respectively, of fibers that incorporate an antimicrobial solution (eg, virucide, bactericide, fungicide) and electrical charges that have been induced during the manufacturing process. As an example, the antimicrobial properties of fibers coated with tea tree and eucalyptus oils have been evaluated against influenza virus, Escherichia coli, Pseudomonas fluorescens, and Bacillus subtilis.\(^\text{53}\) The findings were that E. coli and P. fluorescens were inactivated on the surface of the coated filter within 8 and 2 minutes of exposure, respectively, whereas the more robust B. subtilis was inactivated at a rate of 1 log\(_{10}\) per 30 minutes of process operation. Electrically charged filters are composed of relatively large fibers and characterized by bigger pore sizes than other types of fibrous filters in order to reduce cost and airflow resistance. The capture efficiency of airborne particles is related primarily to the electrostatic charges.

In North America, the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) published a standard to evaluate the performance of commercially available air-cleaning devices as a function of salt particle size entitled Standard 52.2-2012: Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size (https://www.ashrae.org/standards-research—technology/standards-addenda). Per the standard, filter testing is conducted in a test duct at airflow rates between and 1.4 m\(^3\)/s, using particles ranging in size from 0.3 to 1, 1 to 3, and 3 to 10 μm. The overall efficacy is then expressed using the minimum efficiency reporting value (MERV) scale, which ranges from 1-16. As an example, using 0.3- to 1-μm particles, MERV 14 filters have 75%–85% capture efficiency, MERV 15 filters have 85%–95% capture efficiency, and MERV 16 filters have an efficiency >95%. The ASHRAE classification is appropriate for mechanical filters, but does not take into account the microbicidal properties of filters. There is no standard rating for microbicidal or virucidal air-cleaning devices, and studies using ASHRAE standard 52.2 to challenge filters against microbial aerosols are rare.\(^\text{54,55}\)

Air filtration by heating, ventilation, and air conditioning systems equipped with high-efficiency particulate air filters has been shown to ameliorate air quality in hospital rooms and wards.\(^\text{56}\) A few studies have investigated the efficiency of portable high-efficiency particulate air–filtered units in preventing invasive aspergillosis in immunocompromised patients in hospital settings.\(^\text{57}\)

Other methods

Plasma discharge has been tested for the microbiologic decontamination of air and has been shown to be efficient against filamentous fungi.\(^\text{58,59}\) Plasma alters microbes’ viability by charging the particles, making them more prone to capture by electrical filtration. Airlyse technology (Airlyse, France) has been shown to destroy airborne particles in ambient air by denaturing organic compounds by means of UV light and titanium dioxide photocatalysis.\(^\text{59}\) Photocatalysis effectively destroys a wide range of gram-negative and gram-positive bacteria and fungi, algae, protozoa, and viruses.\(^\text{60}\) Several patented devices and technologies claim air decontamination by combining several approaches to filtration and chemical treatment of air (eg, filter exposure to UV radiation on both the upstream and downstream sides and permeation of filters, in situ, with ozone).
Other approaches to reducing the infectious microbial load in the air of indoor environments have been explored. The most studied setting has been the cabins of aircraft, where air contamination is a major concern.63 Several studies have reported transmission of infectious agents during aircraft flights, including influenza,64 measles,65 tuberculosis,66 and severe acute respiratory syndrome.67 Concentrations of selected contaminants in the cabin air of Airbus aircrafts were analyzed, and high-efficiency air filtration, coupled with fresh air dilution, was implemented. Unfortunately, this approach did not prevent the airborne transmission of infectious agents between passengers in aircrafts.68 For this reason, the development of air decontamination systems currently focuses on microbial destruction by photocatalysis, electric shock, or activated carbon fibers.

CONCLUDING REMARKS

Indoor air is increasingly being recognized as a vehicle for a variety of human pathogens. Exposure to airborne pathogens can be via direct inhalation or by contamination of secondary vehicles, such as environmental surfaces. Pathogens on surfaces and objects initially contaminated by air can be resuspended in air for further transport.

The study of human pathogens in air continues to present major challenges, which include the following:

1. Experimental facilities to study the survival and transport of airborne pathogens (viruses, in particular) remain limited because of the need for specialized equipment and appropriate infrastructure and technical skills.
2. Practical and standardized means of recovering viable pathogens from field samples of air also remain unavailable; this prevents us from linking air directly as a vehicle for a variety of infections.
3. Simultaneous or sequential exposure of hosts to airborne pathogens, and other harmful substances, makes risk assessment particularly challenging because of possible combined negative health impacts.
4. Surrogate microbes often used to study the aerobiology of human pathogens may be unsuitable for this purpose because of their inability to withstand aerosolization and remain viable in air. A major research need is identification of better surrogates. Many attributes of bacteriophages make them attractive as surrogates for the study of airborne human pathogenic viruses. This is another topic for further investigation.
5. In spite of the increasing number and variety of technologies claiming indoor air decontamination, robust and scientifically valid protocols remain unavailable for their validation.

Any meaningful approach to addressing the previously mentioned knowledge gaps will require the joint efforts of microbiologists, architects, and specialists in indoor air handling systems.

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