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State of the Science Review

Generic aspects of the airborne spread of human pathogens indoors and emerging air decontamination technologies

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Indoor air can be an important vehicle for a variety of human pathogens. This review provides examples of airborne transmission of infectious agents from experimental and field studies and discusses how airborne pathogens can contaminate other parts of the environment to give rise to secondary vehicles leading air-surface-air nexus with possible transmission to susceptible hosts. The following groups of human pathogens are covered because of their known or potential airborne spread: vegetative bacteria (staphylococci and legionellae), fungi (*Aspergillus*, *Penicillium*, and *Cladosporium* spp and *Stachybotrys chartarum*), enteric viruses (noro- and rotaviruses), respiratory viruses (influenza and coronaviruses), mycobacteria (tuberculous and nontuberculous), and bacterial spore formers (*Clostridium difficile* and *Bacillus anthracis*). An overview of methods for experimentally generating and recovering airborne human pathogens is included, along with a discussion of factors that influence microbial survival in indoor air. Available guidelines from the U.S. Environmental Protection Agency and other global regulatory bodies for the study of airborne pathogens are critically reviewed with particular reference to microbial surrogates that are recommended. Recent developments in experimental facilities to contaminate indoor air with microbial aerosols are presented, along with emerging technologies to decontaminate indoor air under field-relevant conditions. Furthermore, the role that air decontamination may play in reducing the contamination of environmental surfaces and its combined impact on interrupting the risk of pathogen spread in both domestic and institutional settings is discussed.

Air, a universal environmental equalizer, affects all living and nonliving forms on planet earth. For humans, it has profound health implications in all indoor environments where we normally spend most of our time.[1-3] Air quality is also forever changing because of the influence of many controllable and uncontrollable factors that are virtually everywhere. Indoor air, in particular, can expose us to noxious chemicals, particulates, and a variety of infectious agents, as well as pollen and other allergens.[4,5]

Emerging pathogens, such as noroviruses[6] and *Clostridium difficile*,[7] have also been detected in indoor air, with a strong potential for airborne dissemination. Pathogens discharged into the air may settle on environmental surfaces, which could then become secondary vehicles for the spread of infectious agents indoors.[8] The possible transmission of drug-resistant bacteria by indoor air adds another cause for concern.[9] A combination of on-going societal changes is adding further to the potential of air as a vehicle for infectious agents.[10-12] The quality of indoor air is therefore a prominent public health concern[13,14] that requires a clear understanding of the transmission processes for the development and implementation of targeted infection prevention and control measures.[15]

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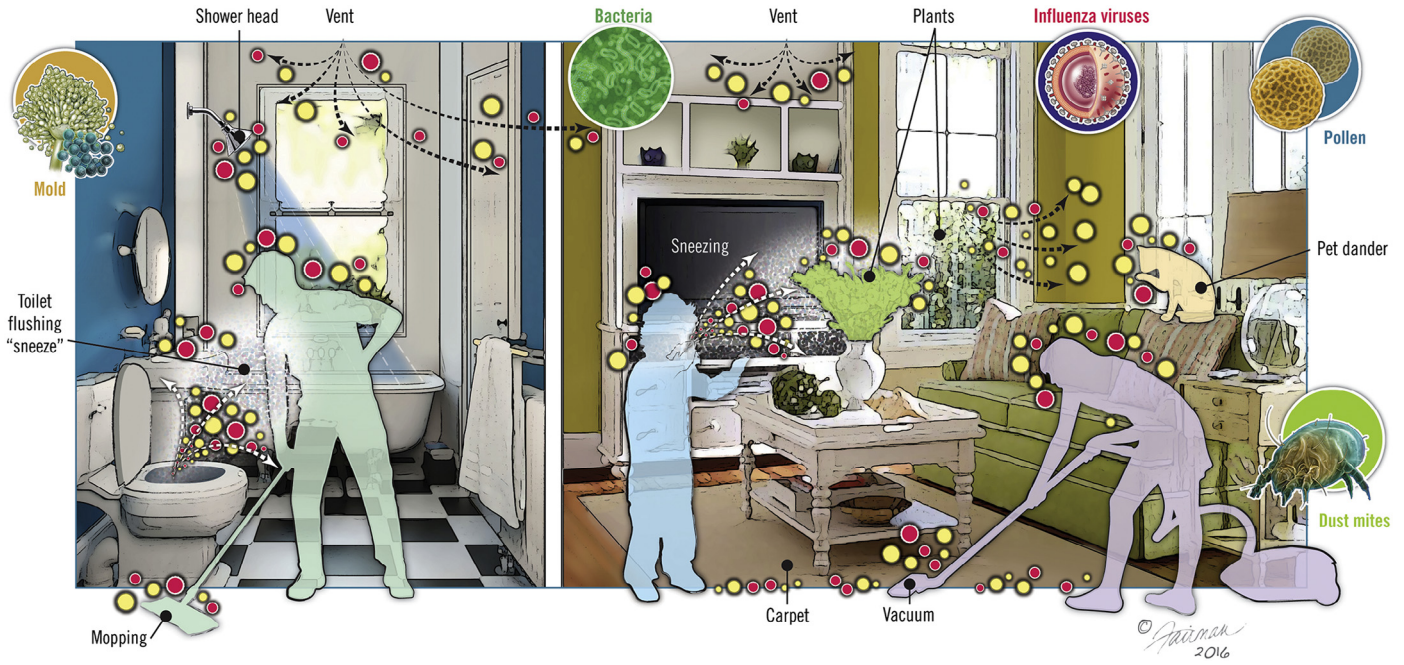

Fig 1. Sources of airborne pathogens indoors and potential for environmental surface contamination. These sources may include humans; pets; plants; plumbing systems, such as operational toilets and shower heads; heating, ventilation, vacuuming, mopping, and air-conditioning systems; resuspension of settled dust; and outdoor air. The yellow and red dots represent human pathogens or harmless microorganisms. Adapted with permission from BioMed Central.[23]

Although direct and indirect exposure to pathogens in the air can occur by other means, infections from the inhalation and retention, including translocation and ingestion after inhalation of droplet nuclei, are generally regarded as true airborne spread. Aerosols of various sizes that contain infectious agents can be emitted from a variety of sources, such as infected or colonized individuals[16] or flushing toilets, and may expose susceptible persons either directly (droplet transmission) or by remaining suspended in the air for inhalation (airborne transmission).[17,18] Contrary to the conventionally held belief, modeling work has redefined the Wells evaporation-falling curve,[19,20] revealing that expelled large droplets could be carried >6 m away by exhaled air at a velocity of 50 m/s (sneezing), >2 m away at a velocity of 10 m/s (coughing), and <1 m away at a velocity of 1 m/s (breathing), leading to potential transmission of short-range infectious agents that contain aerosols.[21]

Airborne transmission requires that pathogens survive the process of aerosolization and persist in the air long enough to be transmitted to a susceptible host.[22] Aerosolized pathogens may settle onto environmental surfaces in the immediate vicinity, leading to genesis of secondary vehicles (Fig 1).[23] This review provides current information on the spread of human pathogens by indoor air, with a focus on the major classes of human pathogens from experimental and field studies, and on emerging air decontamination technologies, including test protocols developed to assess their performance under field-relevant conditions.

METHODS FOR STUDYING AIRBORNE HUMAN PATHOGENS

The study of aerosolized human pathogens requires the ability to produce them experimentally at the appropriate size, store them, and sample them for residual infectious content over a predetermined time period.[13] The equipment must also simulate naturally occurring environmental conditions and the duration of exposure to accurately assess aerosol survivability.[24] Various analytical methods and air samplers have been used to characterize airborne pathogens and overcome the challenges of collecting and analyzing them. Relevant studies have been reviewed in detail elsewhere.[13,25,26]

ENVIRONMENTAL FACTORS THAT INFLUENCE AIRBORNE MICROBIAL SURVIVAL

Aerosolized microbes must survive the prevailing environmental conditions to potentially infect a susceptible host.[22] Multiple factors affect airborne survival of microbes indoors (Table 1).[13,31] The effect of these factors on different types of microbes varies, and generalizations can be difficult because of differences in the experimental methodologies used.[27] Air temperature, relative humidity (RH), and turbulence are among the more important factors affecting the fate and spread of infectious agents indoors.

The analysis of air samples for microbes now includes methods that are based on the polymerase chain reaction (PCR). However, PCR-based methods typically cannot differentiate between viable and nonviable microbes.[32] A recent study found that PCR substantially overestimated the quantity of infectious airborne influenza virus, but the differences in infectious versus noninfectious virus over time were similar to data from quantification by plaque-forming units, which determined that virus losses were evident within 30-60 minutes postaerosolization.[32] Generally, enveloped viruses survive better at lower RH, but there are many exceptions.[28] Other factors that affect aerosol activation in relation to RH include evaporative activity (ie, dehydration, rehydration), surface areas of particles, and pH.[28]

AIRBORNE SPREAD OF MAJOR CLASSES OF HUMAN PATHOGENS

Although studies with experimental animals have determined the susceptibility to airborne pathogens and the minimal infective inhalation dose of a given pathogen,[25] there are wide variations

Table 1
Environmental factors associated with survival of airborne infectious agents[13,26-30]

Environmental factor	Viruses	Bacteria	Fungi
Temperature	• As temperature increases, survival decreases • DNA viruses are more stable than RNA viruses at higher temperatures	• Temperatures >24°C decrease survival	• Highest fungal counts occur in the summer, at higher temperatures
RH*	• Enveloped viruses (most respiratory viruses, influenza) survive longer at lower RH (20%-30%) • Nonenveloped viruses (adenovirus, rhinovirus, and polio virus) survive longer in higher RH (70%-90%) • Exceptionally, nonenveloped rotaviruses survive best at medium RH	• Most gram-negative bacteria survive best in high RH and low temperature, except *Klebsiella pneumoniae*, which is stable at RH 60% • Gram-positive bacteria have the highest death rates at intermediate RH • Sudden changes in RH reduce survival	• Dehydration and rehydration of fungi particles provide conflicting results • Spore concentrations seem higher at higher RH
Atmospheric gases	• Ozone inactivates airborne viruses to a greater degree than bacteria or fungi	• CO decreased survival at low RH (<25%), but protected bacteria at high RH (90%)	• Oxygen supports growth
Light and irradiation	• UV light is harmful (RH-dependent)	• UV light is harmful but may be mitigated by higher RH (water coat protects aerosolized particles)	• More resilient to the effects of UV light than viruses or bacteria
Surrounding organic material (eg, saliva, mucus)	• Protects viruses from environmental changes	• May affect survival based on RH	• Decomposition of organic waste (food remains) may act as a source of fungal spores

CO, carbon monoxide; *RH*, relative humidity; *UV*, ultraviolet.
*RH is a measure of the amount of water vapor in the air at a specific temperature; therefore, temperature and RH always interact to affect survival.

in their test design. First, the number of inhaled microbes may not be known or it may be unrealistically high. Second, the test protocol may not have fully excluded microbial exposure by means other than inhalation. Third, there may be incomplete recording of the environmental conditions (eg, RH, air temperature) to assess their impact on microbial viability. Fourth, pertinent differences may exist between laboratory-adapted strains of the tested microbe compared with strains in the field. Studies using the actual pathogen aerosolized in body fluids provide the strongest evidence of pathogen survivability.[18]

In contrast, field studies face their own set of challenges, which include the noise, bulk, and expense of inefficient air collection devices.[25] Moreover, passive impingers may not adequately collect low concentrations of pathogens found in the clinical environment.[33] Slit sampling does not impose size exclusion and may be more effective at recovering viable pathogens of any size.[33] From a methodologic perspective, field studies also must control for potential variables, such as air turbulence or human activity in areas proximate to sampling, such that sampling occurs before, during, and after an area is occupied and should include functioning ventilation systems.[34]

We have previously reviewed published studies on the airborne spread of viruses of animals and humans.[13,25] Table 2 summarizes key human pathogens with evidence of aerosol transmission. A number of these pathogens causes severe disease, and their classification as high risk by the U.S. Centers for Disease Control and Prevention and the World Health Organization emphasizes the need for appropriate control measures.[18]

Table 2
Key human pathogens with evidence of aerosol transmission[18]

Viruses	Bacteria
Enteric • Norovirus • Rotavirus Respiratory • Hantavirus (Sin Nombre virus) • Influenza virus • Rhinovirus • Coronaviruses (eg, SARS) Neurologic • Rabies virus Skin • Chickenpox • Measles • Mumps • Monkeypox/smallpox	• *Staphylococcus* spp, particularly concerning is MRSA • *Mycobacterium tuberculosis* • *Legionella pneumophila* • *Clostridium difficile* • *Bacillus anthracis*
Fungi	
Aspergillus spp *Penicillium* spp *Cladosporium* spp *Stachybotrys chartarum*	

MRSA, methicillin-resistant *Staphylococcus aureus*; *SARS*, severe acute respiratory syndrome.

Viruses

Experimental studies have used surrogates for human pathogenic enveloped and nonenveloped viruses, such as Cystovirus (ϕ6) and bacteriophage MS-2, respectively.[35] Enteric viruses are transmitted primarily by the fecal-oral route, but airborne transmission has been reported.[13] Airborne transmission of norovirus may be possible via aerosolization of vomitus and toilet flushing, which are regarded as potential sources of both indoor air and environmental surface contamination. Enteric bacteria and viruses have been recovered from indoor air and environmental surfaces in areas surrounding toilets.[18,36,37] We reported that aerosolized simian rotavirus SA-11[38] survived best at midrange RH.[39,40] These results contradicted a prior study by Moe and Harper,[41] in which the UK strain of calf rotavirus was reported to survive best at low and high RH, but not at high temperature.[41] Subsequent studies on human rotavirus,[39] murine rotavirus, and a UK strain of calf rotavirus, aerosolized under the same experimental setup, confirmed the behavior of all strains of rotaviruses are similar in airborne state.[39,40] Furthermore, studies of different picornaviruses (poliovirus type 1 [Sabin] and human rhinovirus) and a human coronavirus (an enveloped virus) that used the same experimental conditions produced results that were consistent with the published literature, suggesting that the experimental design did not introduce bias toward the behavior of aerosolized rotaviruses.[39,42,43]

Among the respiratory viruses, influenza virus is present in the air around infected individuals, and airborne transmission via droplet

nuclei has been demonstrated in experimental models and in reports of influenza spread on-board aircrafts.[15] Low RH favors airborne survival and transmission; however, high air exchange rates facilitate dilution of virus-containing aerosols, regardless of their size.[12] A recent study confirmed recovery of influenza virus from the air emitted by infected persons at distances of 0.5-1.5 m, which could reach the breathing zone of susceptible individuals, including health care workers.[44]

Surprisingly, and in spite of much study, the exact mode of and the relative importance of various types of vehicles for transmission of rhinoviruses, which are the most frequent cause of the common cold, remain shrouded in mystery.[45,46] The behavior of experimentally aerosolized rhinovirus type 14, which represents typical picornaviruses (as previously mentioned),[43] coupled with rhinovirus recovery from both indoor air[47] and outdoor air,[48] substantiate the role of air as a vehicle in spread of some of these picornaviruses. Taken together, an overall assessment of the available evidence suggests a role for airborne spread[49] and for the role of contaminated hands and environmental surfaces in rhinovirus dissemination.[50]

Coronaviruses are the second leading cause of the common cold and are also responsible for the severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome.[37] SARS is thought to be transmitted via direct contact, but airborne transmission is also suspected because the virus has been detected in air samples that were collected from rooms where a patient was recovering from SARS.[18,33] The virus is spread through droplets and can remain viable on surfaces for several days at room temperature. The use of aerosol-generating procedures, such as intubation, bronchoscopy, and oxygen delivery vents, may promote dispersal of SARS via enhanced release in mists of exhaled pulmonary gases.[34,51] Our earlier work on the behavior of aerosolized human coronaviruses 229E further substantiates the potential role of air in their aerial spread.[39]

Aerosol transmission of the Ebola virus is biologically plausible.[18] The virus is present in saliva, stool, blood, and other body fluids; therefore, it could be aerosolized through symptoms associated with infection or via health care procedures. The Ebola virus has been shown to survive in the air when the half-life of the virus ranged from 15 (Zaire Ebola virus) to 24 minutes (Reston Ebola virus), and the time for 99% biologic decay of the aerosolized virus held in rotating drum (at 50%-55% RH and 22°C ± 3°C) was estimated to be between 104 and 162 minutes. Additionally, infection of rhesus monkeys via experimentally aerosolized Ebola virus has also been reported.[52] These findings raise concerns for aerosol transmission and control of this serious pathogen; however, thus far, there is no clear evidence for the airborne spread of this virus in humans.[18] Epidemiologic evidence indicates transmission is associated with direct physical contact or contact with body fluids; however, the possibility of aerosolized spread has been postulated by Ebola virologists.[18,53]

Bacteria

Approximately one-third of humans carry *Staphylococcus aureus*, with the anterior nares as a common site of colonization,[54] and environmental contamination plays an important role in the transmission of methicillin-resistant *S aureus*. Shedding of the bacteria is highly variable, but transmission likely occurs via skin squames that settle out on environmental surfaces in the vicinity. Smaller particles may remain airborne, particularly if there is air turbulence.[54,55] An important characteristic of the staphylococci is their ability to survive over a wide range of temperatures, RH, and exposure to sunlight.[54]

Mycobacterium tuberculosis is transmitted via droplet nuclei expectorated from infected persons during coughing, sneezing, and talking.[18,34] Control measures include expensive negative-pressure ventilation and less expensive, but climate-dependent, natural ventilation.[56] Upper-room ultraviolet (UV) light or negative air ionization may help reduce the airborne spread of *M tuberculosis*.[56]

Nontuberculous mycobacteria are found in soil and water sources and can form biofilms under domestic environments, such as shower heads.[57] Transmission to humans is uncertain, but droplet aerosolization is a suspected route of pulmonary disease, with shower heads considered a common source.[57,58] Contamination of hospital water supplies and medical equipment are suspected in nosocomial outbreaks of disease.[59] Similarly, *Legionella* spp become airborne by active aerosolization of contaminated water and form biofilms in air conditioning systems.[34] *Legionella*-like amoebal pathogens are a subset of bacteria that grow within amoebae and often are coinfectious agents with other bacteria and fungi.[60]

Clostridium difficile spores have been recovered from the air near symptomatic patients, especially those with recent-onset diarrhea.[61] Air samples were positive for *C difficile* in 70% of patients, and the highest levels of surface recovery were in areas closest to the patient.[7] The isolates recovered from the air were indistinguishable from those recovered from fecal samples and from the environment in the same settings.[7] Additionally, *C difficile* has been recovered after toilet flushing, and leaving the lid open when flushing increases contamination of surrounding environmental surfaces.[61]

Airborne infection of *Bacillus anthracis* is affected by environmental factors that include room size, ventilation rate, and host factors, such as pulmonary ventilation rate.[62] Secondary aerosolization of viable *B anthracis* spores was reported after contamination of a U.S. Senate office, with >80% of particles being in the respirable size range of 0.95-3.5 μm.[14]

Fungi

As ubiquitous microorganisms, fungi pose a health threat in indoor environments.[29] Fungal infections can be particularly serious in immunocompromised patients,[63] especially airborne spores of *Aspergillus* spp that are blown in from natural ventilation sources.[27] Fungal spores are aerosolized from municipal water supplies and dust and can be effectively transported over long distances by wind and air currents.[63,64] The evolution of the fungal spore has enabled them to travel long distances and be more capable of withstanding environmental insults.[27] The most important factor of fungal growth in indoor environments is humidity[65]; therefore, control measures include dehumidification of the air and high-efficiency particulate arrestor filtration.[27]

Recent research suggests that airborne fungal particles are heterogeneous and comprise spores and submicrometer fragments.[66,67] These fragments are of significant interest with regard to health because they remain in the air longer and are easily inhaled. There are also a variety of fungal components that have been identified in air, including mycotoxins, ergosterols, glucans, and microbial volatile organic compounds, and these require unique analysis methods.[64] Taken together, these findings provide a foundation for the definition of sick building syndrome.[68] High humidity within sick houses and buildings allows for growth of fungi indoors, particularly species of *Aspergillus*, *Penicillium*, and *Cladosporium* and *Stachybotrys chartarum*, an indoor mold that was associated with sick building syndrome several decades ago.[64,68,69] These fungi can be found in dust, furniture, carpets, and ventilation systems at concentrations ranging from 0-1,000 colony-forming unit (CFU)/m^3.[64] In fact, carpet has been described as a sink for fungi, but it is also a source for resuspension of fungal particles into the air.[64] Various respiratory conditions (eg, wheeze, cough, asthma) have been linked

Table 3
Current and emerging technologies for decontamination of indoor air for human pathogens

Technology	Description	Pathogen tested	Remarks
UV irradiation			
Microgenix air purification system	Chemical-coated filter and UV source for reducing microbes in HVAC systems	Aerosolized MS-2 phage as surrogate for viruses	• Inactivation efficiency = 97.34% with UV, 61.46% without UV
Upper-room 254 nm UVC light	Exposure to UV light (254 nm) field separated by manifolds at 4 levels of temperature and RH	Porcine reproductive and respiratory syndrome virus	• Virus was most susceptible to UV 254 nm as temperature decreased and RH was between 25% and 79%
	UV light (254 nm) at 3 levels of RH	Influenza A virus (H1N1, PR-8)	• Virus susceptibility to UV increased with decreasing RH
	UV light (254 nm) under real-world conditions of convection, mixing, temperature, and RH	Vaccinia virus as a surrogate for smallpox	• Virus susceptibility to UV increased with decreasing RH • Virus susceptibility did not appear to be a function of aerosol particle size
	UV light (254 nm)	Respiratory adenovirus, murine hepatitis virus, a coronavirus as surrogate for SARS, and bacteriophage MS-2	• Adenovirus and MS-2 were resistant to UV decontamination • High RH did not protect viral aerosols
UV germicidal irradiation (8 lamps emitting peak 253.7 nm UVC light)	Airborne virus was passed through a cylinder that was 0-30 cm from UV source	Four bacteriophages (a single strand each of RNA and DNA and a double strand each of RNA and DNA)	• Single-strand viruses were more susceptible to UV and inactivation occurred to a greater degree at higher RH
Oxygen-based technologies			
Hydroxyl/Odorox product	Claims to inactivate all types of pathogens on surfaces and in the air		• No published references; only Web site (www.eairsolutions.com/pbenefits.htm)
Phocatox	Combination of HEPA filtration, hydroxyl radical production, purified O_3, and vaporized gas-phase hydrogen peroxide plus UVC	Claims to decontaminate air and surfaces of a wide range of pathogens—viruses, bacteria (including MRSA and *Clostridium difficile*), and fungi	• No published references; Web site (www.phocatox.com/index.htm)
TriAir T250	Hydroxyl radicals	Gram-positive and gram-negative bacteria; enveloped and nonenveloped viruses	• No published references; only Web site (www.tri-airdevelopments.co.uk)
Inov8 Air Disinfection unit	Hydroxyl radicals	All types of pathogens	• No published references; Web site (www.inov8.com)
Ozone generator	Gaseous ozone and aerosolized virus were generated continuously into the chamber	Bacteriophages: single-strand RNA and DNA, double-strand RNA and DNA	• 95% of virus aerosol was <2.1 μm in diameter • More complex virus capsid was less susceptible to ozone • Viruses were more susceptible to ozone at higher RH
Cold oxygen plasma	Viruses nebulized into tunnel with phosphate-buffered saline	Human parainfluenza virus-3, respiratory syncytial virus, influenza virus H5N2	• Technology has potential as long as ozone levels are safe
Nonthermal plasma reactors	Air flows in near the floor and is filtered with plasma and exhausted from top	H5N2 avian flu strain as surrogate for H1N1	• 4- to 5-log reduction after a single pass • Similar performance at temperatures 10°C-40°C and RH up to 98%
Sharp air purifier	Combination of plasmacluster ion technology and multiple layers of filtration	Bacteria and viruses	• No published references; Web site (www.sharp.ca/en-CA/Forhome/HomeEnvironment/AirPurifier.aspx?)

HEPA, high-efficiency particulate arrestor; *HVAC*, heating, ventilation, air conditioning; *MRSA*, methicillin-resistant *Staphylococcus aureus*; *RH*, relative humidity; *SARS*, severe acute respiratory syndrome; *UV*, ultraviolet; *UVC*, energy-rich ultraviolet light with a wavelength of 200-400 nanometers (nm). Adapted with permission from Elsevier.[25]

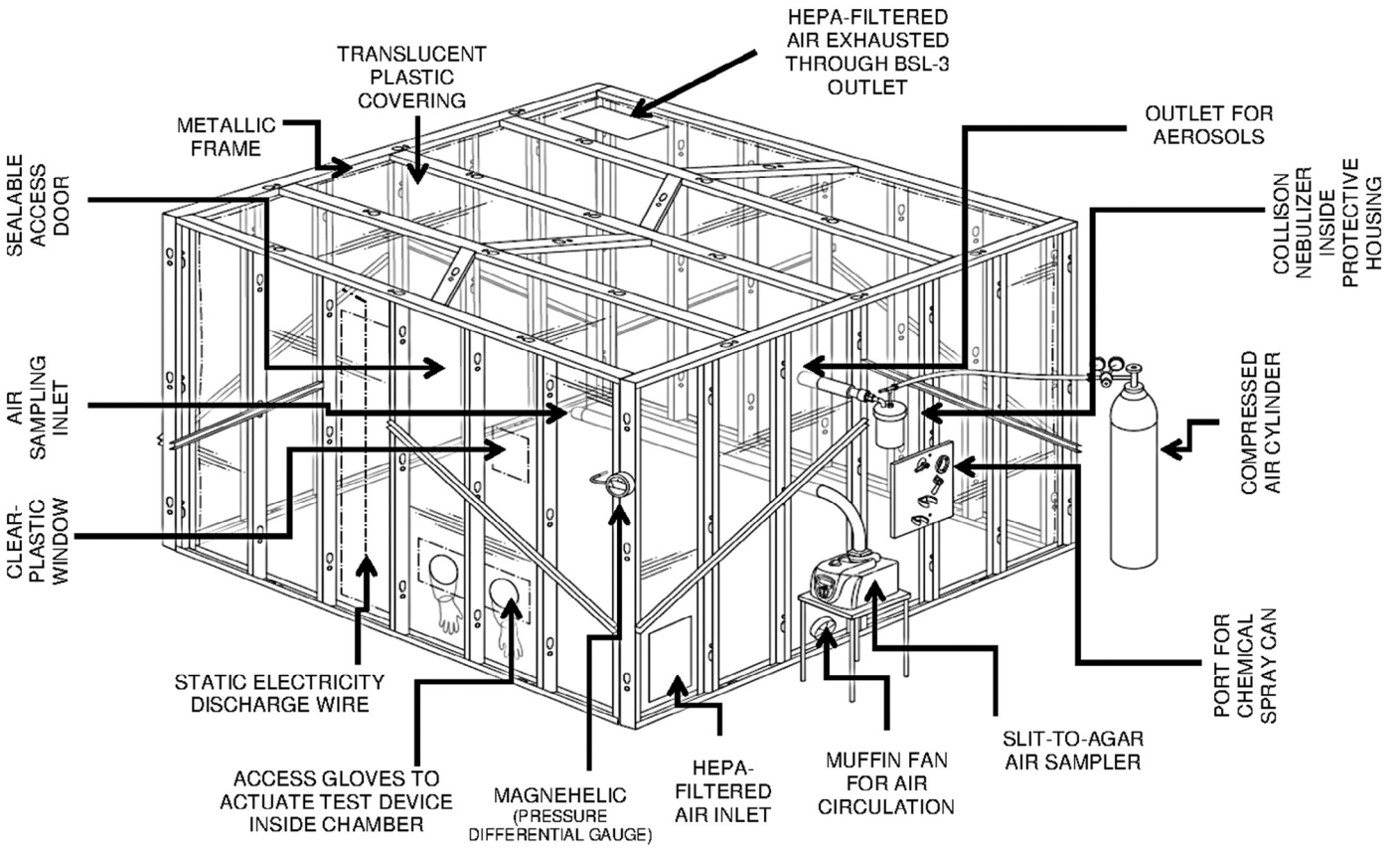

Fig 2. Aerobiology chamber with essential components (length × width × height: 320.0 × 360.6 × 211.0 cm—24.3 m^3 [860 ft^3]). Reprinted with permission from Elsevier.[73]

to fungi and their biologic components in the indoor environment.[64] Fungal species found outdoors include *Cladosporium* and *Alternaria* spp, which are responsible for triggering hypersensitivity reactions, including rhinitis, sinusitis, and asthma.[27,64]

CURRENT AND EMERGING AIR DECONTAMINATION TECHNOLOGIES

The clear recognition of indoor air as a vehicle for pathogens has incurred a corresponding upsurge in the marketing of products and technologies with claims for safe and effective decontamination of air.[70] Although many technologies are available for environmental surface decontamination, the number and variety of those for decontamination of indoor air remain limited and of questionable veracity (Table 3). The air-decontaminating claims of many such technologies are not based on testing under field-relevant conditions with pathogens relevant to human health, and scientifically valid and standardized protocols to generate field-relevant data for label claims for review by regulatory and public health agencies and the public at large remain unavailable. Here, we address this gap in the development of a test platform for standardized testing of commercially available devices for decontaminating indoor air of vegetative bacteria that represent airborne human pathogens. We know of only one guideline that directly relates to this topic.[71] It specifies the size of a sealed enclosure for experimental contamination of the air with aerosols of vegetative bacteria to assess technologies for their temporary reduction. Therefore, the text that follows relates directly to that guideline.

BASIC EXPERIMENTAL DESIGN AND OPERATION OF AN AEROBIOLOGY CHAMBER

The studies of microbial survival in indoor air, as well as proper assessment of methods for its decontamination, emphasize numerous challenges and highlight the need for specialized equipment and protocols. Proper expertise and suitable experimental facilities for such investigations remain uncommon. Several of the available sites with testing claims are neither experienced in, nor equipped to conforming with, the U.S. Environmental Protection Agency's (EPA) guidelines on testing the sanitization of indoor air.[71] Based on our considerable experience in the study of airborne human pathogens,[13,25,39,43,72] we have built an aerobiology chamber (Fig 2) designed to meet the requirements of the EPA guidelines and have used this to study the effects that a variety of air decontamination technologies have on the airborne survival and inactivation of vegetative bacteria, viruses (bacteriophage), and bacterial spore-formers (Sattar et al, unpublished data). Additional details about the operational aspects of the aerobiology chamber, described elsewhere,[73] are discussed briefly.

EXPERIMENTS USING THE AEROBIOLOGY CHAMBER

Testing microbial survival

Any meaningful assessment of air decontamination requires that the aerosolized challenge microbe remain viable in the experimentally contaminated air long enough to allow for proper differentiation between its biologic decay or physical fallout and inactivation or removal by the technology being assessed. There-

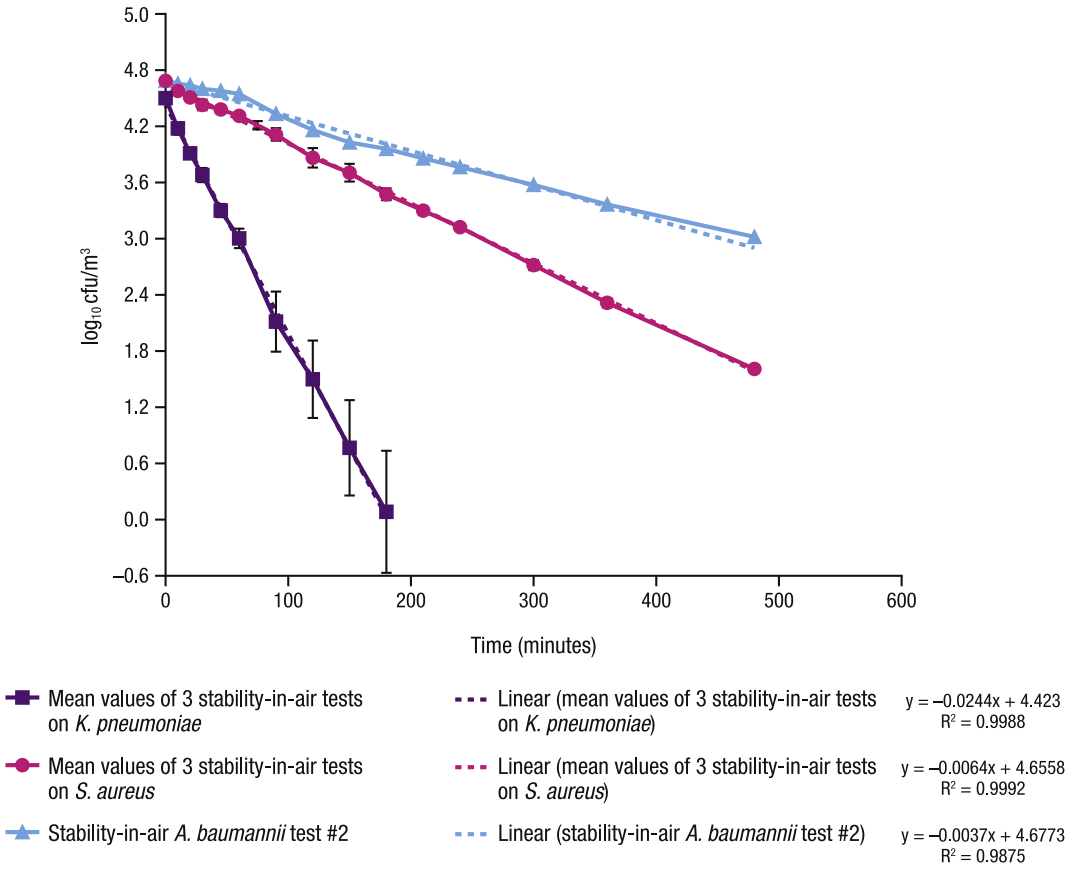

Fig 3. Comparative rates of biologic decay of aerosolized *A. baumannii*, *S. aureus*, and *K. pneumoniae* held within the aerobiology chamber. *A. baumannii*, *Acinetobacter baumannii*; *cfu*, colony forming units; *K. pneumoniae*, *Klebsiella pneumoniae*; *S. aureus*, *Staphylococcus aureus*.

Table 4
Specifications of 3 devices that were tested for their ability to decontaminate experimentally aerosolized microbial challenge within the aerobiology chamber

Device no.	Flow rate, ft^3/min (m^3/min)	Time to expose entire contents of the chamber once	Theoretical no. of exposures of an aerosol particle in 8 h	UV light bulb wattage
1	100 (2.831)	0.143 h (8.594 min)	55.94	5 (LB 4000)
2	120 (3.398)	0.12 h (7.16 min)	66.67	8 (LB 5000)
3	60 (1.699)	0.239 h (14.32 min)	33.47	9 (ZW6S12W)

UV, ultraviolet.

fore, initial testing is required to determine the rate of biologic decay of the test microorganism(s) under the experimental conditions to be used for testing potential air decontamination technologies. For this, the test microorganism(s) was aerosolized into the chamber, and 2-minute air samples were collected at different intervals using a slit-to-agar (STA) sampler over an 8-hour period. The culture plates were incubated at 36°C ± 1°C, the CFU on them was recorded, and the data were analyzed to determine the rate of biologic decay.

The results of the tests on the airborne survival of 3 types of vegetative bacteria are shown in Figure 3. *Acinetobacter baumannii* (ATCC 19606; ATCC, Manassas, VA) proved to be the most stable in air, followed by *S aureus* (ATCC 6538; ATCC) and *Klebsiella pneumoniae* (ATCC 4352; ATCC).

Testing of 3 types of indoor air decontamination devices

Three types of commercially available indoor air decontamination devices that were based on UV light and high-efficiency particulate arrestor filtration were tested for their ability to reduce the levels of viable bacteria in the air of the chamber (Table 4). The air within the chamber was first experimentally contaminated with aerosolized test bacterium suspended in a soil load. The test device, placed inside the chamber, was remotely operated, and samples of the chamber air were collected directly onto Petri plates using STA and were incubated for CFU determinations.

As shown in Figures 4A and 4B, the air decontamination devices that were tested could achieve a 3-$\log_{10}$ reduction in viability of *S aureus* and *K pneumoniae* in 38-45 minutes (Table 5). So far, such testing has been conducted only once with *A baumannii* using device 1, and as the data presented in Figure 5 show, it reduced the viability of *A baumannii* by 3 $\log_{10}$ in 38 minutes (Table 5).

Testing with repeated microbial challenge

In this experiment, device 1 was tested for its ability to manage ongoing fluctuations in the microbiologic quality of indoor air. A suspension of *S aureus* was nebulized into the chamber at 3 separate time points, while the device operated continuously. As shown in Figure 6, the device's efficacy after the 3 challenges with

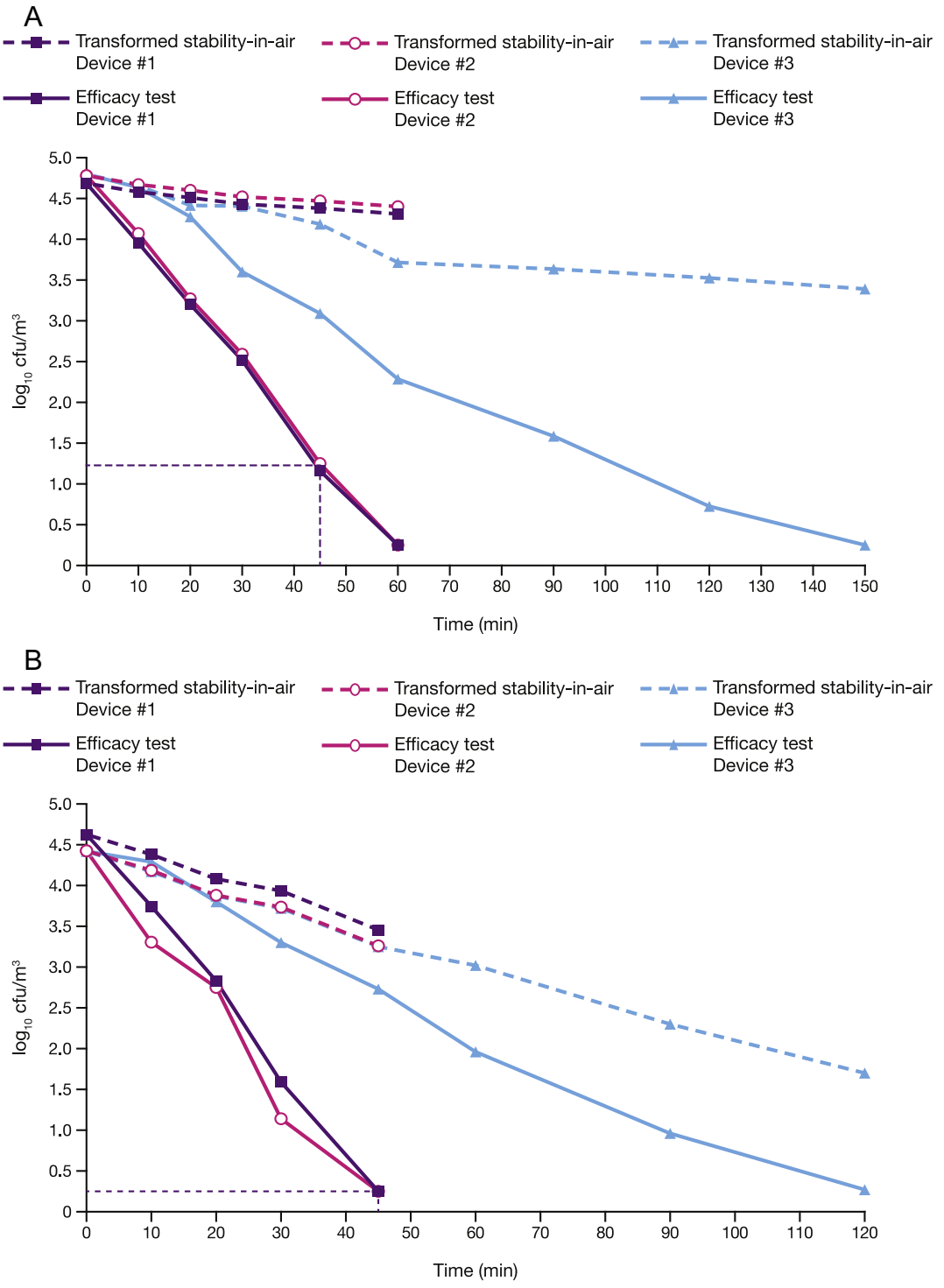

Fig 4. (A) Comparative inactivation rates of airborne *Staphylococcus aureus* during the operation of 3 indoor air decontamination devices. *cfu*, colony forming units. Reprinted with permission from Elsevier.[73] (B) Comparative inactivation rates of airborne *Klebsiella pneumoniae* during the operation of 3 indoor air decontamination devices. *cfu*, colony forming units. Reprinted with permission from Elsevier.[73]

aerosolized bacteria was almost the same. The times at which the device demonstrated 3-$\log_{10}$ reductions after each nebulization were found to be 40, 39.5, and 40.9 minutes. The mean of the 3-$\log_{10}$ reduction times was 40.13 ± 0.71 minutes, giving an average biologic decay rate of aerosolized bacteria of 0.0753 ± 0.0024 CFU/m^3/min after the 3 nebulizations.

Reducing microbial contamination of environmental surfaces by inactivation of airborne vegetative bacteria

As previously mentioned, larger particles of aerosolized pathogens often settle onto environmental surfaces in the immediate vicinity, leading to contamination as a secondary vehicle of trans-

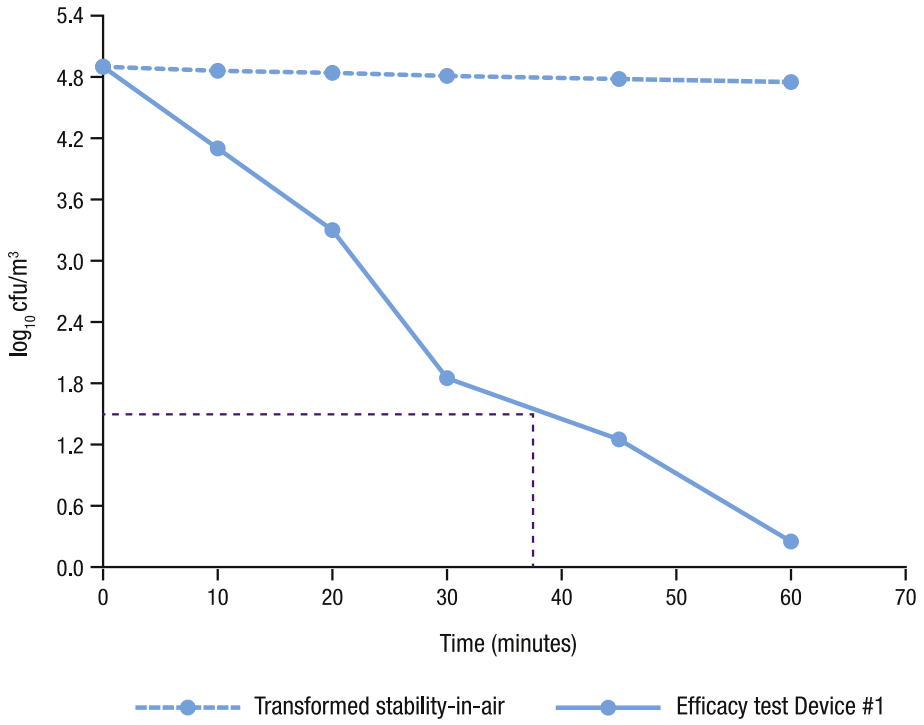

Fig 5. Inactivation of aerosolized *Acinetobacter baumannii* during operation of an indoor air decontamination device (device 1). *cfu*, colony forming units.

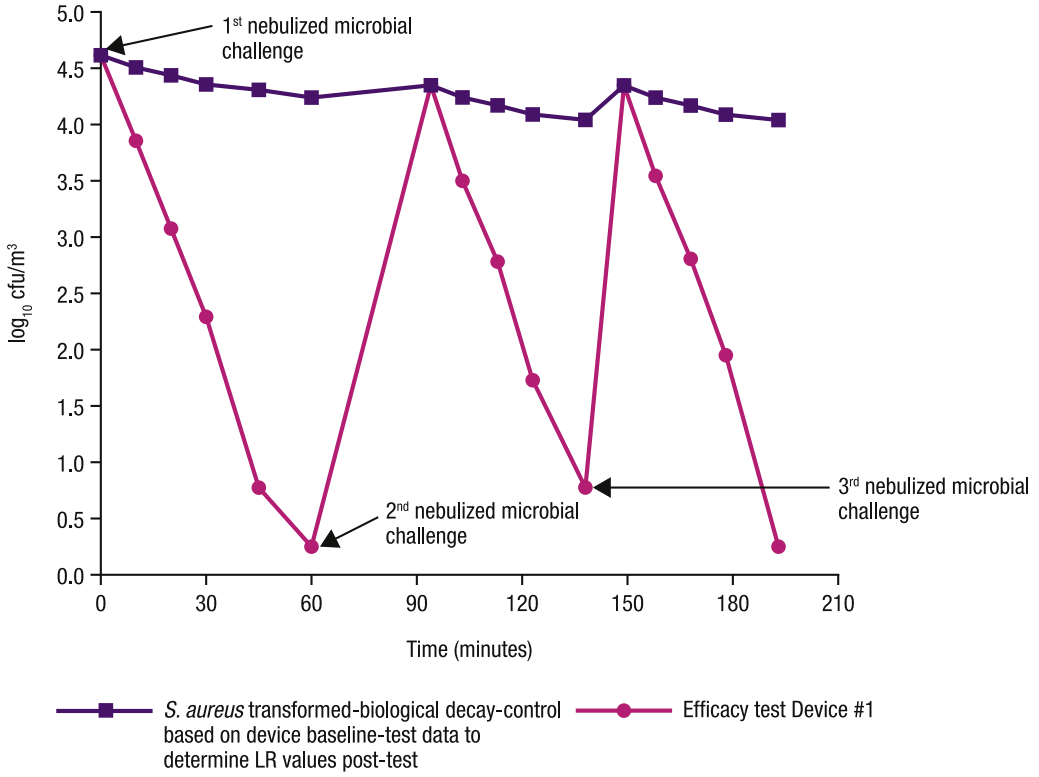

Fig 6. Repeated microbial challenge with aerosolized *S. aureus* during operation of device 1. *cfu*, colony forming units; *LR*, log10 reduction; *S. aureus*, *Staphylococcus aureus*.

mission, or reaerosolize through human or mechanical activity, such as vacuuming. To determine if targeting aerosolized microorganisms could reduce the contamination of environmental surfaces, device 1, with proven efficacy against airborne vegetative bacteria, was tested to see if it could also reduce the level of contamination of environmental surfaces in the same setting. *S aureus* (ATCC 6538; ATCC) was used as the challenge microbial aerosol, and surfaces were disposable plastic Petri plates (100 mm in diameter).

Table 5
Regression coefficients, *P* values comparing decay rates of efficacy tests with stability in air, and times required to achieve 3 $\log_{10}$ reductions

Device	Slope			Intercept			*P* value			≥3 $\log_{10}$ reduction (min)		
	S aureus	*K pneumoniae*	*A baumannii*	*S aureus*	*K pneumoniae*	*A baumannii*	*S aureus*	*K pneumoniae*	*A baumannii*	*S aureus*	*K pneumoniae*	*A baumannii*
Stability in air	−0.0064	−0.0244	−0.0037	4.6471	4.4657	4.8829	NA	NA	NA	NA	NA	NA
Efficacy test device 1	−0.0752	−0.099	−0.079	4.6977	4.6871	4.7821	3.82×10^{-10}	2.93×10^{-5}	NA	45	45	38
Efficacy test device 2	−0.0766	−0.0983	ND	4.8243	4.5763	ND	1.39×10^{-9}	2.00×10^{-4}	NA	45	45	ND
Efficacy test device 3	−0.0224	−0.0369	ND	4.9301	4.4449	ND	2.13×10^{-10}	2.93×10^{-5}	NA	215	215	ND

A baumannii, *Acinetobacter baumannii*; *K pneumoniae*, *Klebsiella pneumoniae*; *NA*, not applicable; *ND*, not done; *S aureus*, *Staphylococcus aureus*.

Fifteen sterile plastic plates were placed in groups of 3 on the floor of the aerobiology chamber, with one set in each of the 4 corners and one in the center. The lids of the plates were removed. A suspension of *S aureus* in a soil load was nebulized into the chamber with the muffin fan operating for 5 minutes to evenly distribute the airborne bacterial particles. A 2-minute air sample was then collected from the chamber using an STA sampler to determine the initial level of airborne contamination. Ten minutes were allowed to elapse for circulation of the airborne bacteria in the chamber. The muffin fan was then turned off and the airborne bacteria were allowed to settle for 30 minutes. At the end of this period, the Petri plates were retrieved and eluted for CFU to determine the titer of microbial contamination deposited on each one. Such testing allowed us to determine the levels of airborne bacteria that could settle on the plates without air decontamination procedures.

The experiment was repeated in exactly the same manner, but with the test device in the chamber activated and allowed to work for 45 minutes. At the end of this period, the Petri plates were retrieved and eluted for CFU to determine the titer of microbial contamination deposited on each plate.

The results indicated that the nebulization of the microbial suspension for 10 minutes produced 4.7 $\log_{10}$ CFU/m^3 of air in the chamber. The average level of CFU on the control and test Petri plates held in the chamber was 200 ± 110 and 8.3 ± 8.9, respectively. The device could reduce the contamination of the plates from airborne bacteria by 95% as compared with the controls.

DISCUSSION AND CONCLUDING REMARKS

Recognition that human pathogens can be transmitted via indoor air emphasizes the need for the development of control procedures that limit exposure and reduce the risk of infection in susceptible individuals. This need is heightened by an increase in the aging population and numbers of the immunosuppressed. We must also be prepared for an intentional or accidental release of infectious aerosols. Standardization of sampling and analytical methods is crucial to developing an understanding of airborne pathogens[26] and technologies for their effective control.

We have described the creation and application of an aerobiology test chamber that complies with the relevant guideline of the EPA.[71] The chamber was successfully used (1) to study the airborne survival of 3 types of vegetative bacteria under ambient conditions; (2) to test the ability of 3 commercial indoor air decontamination devices to abate experimentally generated aerosols of 3 types of vegetative bacteria; (3) to test one of the devices for its ability to deal with repeated microbial challenge with vegetative bacteria in simulation of situations in which indoor air is contaminated on an on-going basis; and (4) to test one of the air decontamination devices for its effectiveness in reducing the level of microbial contamination of environmental surfaces as a function of reducing airborne bacteria.

Each of these experiments was completed successfully, thereby demonstrating the suitability of the aerobiology chamber and the protocols for aerosol generation and sampling. The use of the STA sampler proved particularly effective for providing event-related information on the levels of viable bacteria in the air of the chamber.

The testing with *A baumannii* clearly demonstrated that it is more suitable than *K pneumoniae* as a surrogate for gram-negative bacteria. *A baumannii* is not only a relevant airborne pathogen that is more resistant to aerosolization, but it also is more stable in the airborne state.[74] Therefore, it is recommended that it be considered as an alternative for *K pneumoniae* by regulatory agencies, such as the EPA, for testing and registration of air decontamination technologies.

The experimental facility and test protocols described here are suitable for work with other types of airborne human pathogens, such as viruses, fungi, and bacterial spore formers. The aerobiology chamber also could be readily adapted to assess emerging technologies of indoor air decontamination. Although the work reported here was performed in a sealed and empty chamber, as specified in the EPA guidelines, the aerobiology chamber can be modified to represent air exchanges, and furniture can be introduced to simulate a typical room under both domestic and institutional settings.

Air, in general, is crucial to the establishment and maintenance of the indoor microbiome, and the continual redistribution of microbes indoors occurs at the air-surface-air nexus. Although classic airborne spread of pathogens occurs via droplet nuclei, droplets can potentially contaminate environmental surfaces, depending on their size and prevailing environmental conditions, thereby creating secondary vehicles for pathogens. Therefore, targeting airborne pathogens could potentially provide an additional advantage by reducing environmental surface contamination. Our preliminary findings indicate that a reduction in the level of viable airborne bacteria using active air decontamination can also reduce bacterial contamination on environmental surfaces in the same setting. Therefore, targeting airborne pathogens could entail additional benefits, such as preventing or reducing the deposition of harmful microbes on secondary vehicles that include frequently touched environmental surfaces and also preventing or reducing their resuspension from these surfaces back into the air via a variety of indoor activities (Fig 1).[8,12,17,75,76] Further studies should investigate the role air decontamination may play in reducing the contamination of environmental surfaces and its combined impact on interrupting the risk of pathogen spread in both domestic and institutional settings.

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