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Commentary

Indoor air as a vehicle for human pathogens: Introduction, objectives, and expectation of outcome

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Key Words:
Aerobiology
indoor air
airborne pathogens
air decontamination
airborne pollutants

Airborne spread of pathogens can be rapid, widespread, and difficult to prevent. In this international workshop, a panel of 6 experts will expound on the following: (1) the potential for indoor air to spread a wide range of human pathogens, plus engineering controls to reduce the risk for exposure to airborne infectious agents; (2) the behavior of aerosolized infectious agents indoors and the use of emerging air decontamination technologies; (3) a survey of quantitative methods to recover infectious agents and their surrogates from indoor air with regard to survival and inactivation of airborne pathogens; (4) mathematical models to predict the movement of pathogens indoors and the use of such information to optimize the benefits of air decontamination technologies; and (5) synergy between different infectious agents, such as legionellae and fungi, in the built environment predisposing to possible transmission-related health impacts of aerosolized biofilm-based opportunistic pathogens. After the presentations, the panel will address a set of preformulated questions on selection criteria for surrogate microbes to study the survival and inactivation of airborne human pathogens, desirable features of technologies for microbial decontamination of indoor air, knowledge gaps, and research needs. It is anticipated that the deliberations of the workshop will provide the attendees with an update on the significance of indoor air as a vehicle for transmitting human pathogens with a brief on what is currently being done to mitigate the risks from airborne infectious agents.

> "Clean air is a basic requirement of life. The quality of air inside homes, offices, schools, day care centres, public buildings, health care facilities or other private and public buildings where people spend a large part of their life is an essential determinant of healthy life and people's well-being. . ." –World Health Organization, 2010

I welcome you all to this multinational workshop! This workshop was conceived over a year ago, and the organizing committee (Table 1) formally requested that ASTM International (www.astm.org/) hold the event under its auspices. ASTM's Committee E35, which deals with pesticides, antimicrobials, and alternative control agents, approved the proposal in April 2015.

Mounting recognition of indoor air as a vehicle for infectious agents is leading government regulators, such as the U.S. Environmental Protection Agency, to refine and update their guidelines,[1] researchers to develop better means of studying airborne pathogens,[2] and civil engineers and architects to find innovative means of making indoor air safer while keeping energy conservation in mind.[3]

Although comprehensive guidelines and standardized means are available to study chemical pollutants in indoor air,[4] there remains a general lack of suitable experimental facilities and standardized protocols to quantitatively assess the survival of pathogens in indoor air and to document their removal and inactivation by physical and chemical means. This workshop will address these issues, among others.

SPECIFIC OBJECTIVES

The workshop's specific objectives, therefore, are as follows:

- To provide a forum for the exchange of ideas on current research on the role of the indoor environment in general and indoor air in particular in the spread of human pathogens;

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Conflicts of Interest: None to report.

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Table 1
Workshop organizing committee and support staff

Designation	Name	Affiliation
Chair	John A. Mitchell	Wordsmith Scientific and Regulatory, LLC, 3304 Wagon Wheel Rd, Bozeman, MT 59715
Co-chair	M. Khalid Ijaz	RB, One Philips Pkwy, Montvale, NJ 07645 and Adjunct Associate Professor of Biology, Medgar Evers College of The City University of New York (CUNY), Brooklyn, New York
Secretary	Mary K. Bruch	Mary Bruch Micro Reg Inc, 23 Hamilton Terrace, Hamilton, VA 20158
Members	Absar Alum	Arizona State University, Tempe, AZ 85281
	Elizabeth (Ilze) Bruning	R&D Manager, Strategic Development & Clinical Hygiene—Germ Protection & Personal Care, RB, One Philips Pkwy, Montvale, NJ 07645
	Tony Buhr	CBR Concepts and Experimentation, 4045 Higley Rd, Ste 345, Dahlgren, VA 22448
	Delbert Harnish	Research Microbiologist, Applied Research Associates, Engineering Science Division, 430 W 5th St, Ste 700, Panama City, FL 32401
	Brian Heimbuch	Associate Division Manager/Senior Bioaerosol Scientist, Applied Research Associates, Engineering Science Division, 430 W 5th St, Ste 700, Panama City, FL 32401
	Syed A. Sattar	Professor Emeritus of Microbiology, Faculty of Medicine, University of Ottawa, Ottawa, Canada
	Alice Young	CBR Concepts and Experimentation, 4045 Higley Rd, Ste 345, Dahlgren, VA 22448
Coordinators*	Jennifer Rogers	Symposia Operations, ASTM International, 100 Barr Harbor Dr, West Conshohocken, PA 19428
	B. Milewski	Staff Responsible for Committee E35, ASTM International, 100 Barr Harbor Dr, West Conshohocken, PA 19428
	Kelly Dennison	Symposia Operations, ASTM International, 100 Barr Harbor Dr, West Conshohocken, PA 19428
Administrative assistant	Hannah Sparks	Symposia Operations, ASTM International, 100 Barr Harbor Dr, West Conshohocken, PA 19428
Recording secretaries	Kathy Baxter, Darla Goeres, Rhonda Jones, and Carol Vincent	Members, ASTM Subcommittee E35.15, ASTM International, 100 Barr Harbor Dr, West Conshohocken, PA 19428

*Mary Mikolajewski and Ellen Diegel served as coordinators for ASTM in the early stages of the workshop planning.

- To discuss experimental facilities and test protocols for the study of airborne microbial survival;
- To review available means of recovering viable microbes from indoor air;
- To propose better surrogates for the study of indoor air as a vehicle for human pathogens;
- To model microbial movements in indoor air for further improvements in the design of experimental aerobiological facilities and test protocols; and
- To review ongoing research on physical and chemical means of indoor air decontamination.

The deliberations will also focus on the development of standards for assessing indoor air decontamination technologies and government regulations for registration of products to be marketed.

SPONSORSHIP AND FINANCIAL SUPPORT

As noted, this workshop has been organized under the auspices and with the support of ASTM International. The City University of New York and the University of Ottawa (Canada) are the 2 academic sponsors of the workshop, and financial support has been provided by RB (Montvale, NJ) and Microbac (Sterling, VA). These 2 companies are also funding publication of the workshop proceedings. We gratefully acknowledge their generous support.

THE PROGRAM

The organizing committee has put together an outstanding group of speakers who will offer a comprehensive yet balanced perspective on the key issues. Table 2 lists the topics to be covered, along with the names and affiliations of the presenters.

WORKSHOP PROCEEDINGS

Elsevier (www.elsevier.com) has agreed to publish the proceedings of the workshop after peer review. Elsevier will also provide a preview of the proceedings, including the abstracts for each presentation, for release during the 2016 conference of the Association for Professionals in Infection Control and Epidemiology. The workshop proceedings will also contain a summary of the concluding discussions.

TARGET AUDIENCE

Potential members of the audience include researchers in aerobiology, makers of air purification technologies, contract laboratories that assess air decontaminants, government regulators dealing with indoor air quality, and members of standards-setting organizations, such ASTM International (www.astm.org) and American Society of Heating, Refrigerating, and Air-Conditioning Engineers (www.ashrae.org).

BACKGROUND ON THE TOPIC

Table 3 is a glossary of the common terms used throughout this workshop's presentations. This is included in an attempt to create a level playing field while facilitating the understanding of the subject matter by experts in fields other than environmental microbiology. However, the emphasis here is on working definitions, recognizing that efforts are needed to develop a more comprehensive glossary for broader applications in this area.

Aerobiology

Aerobiology, the study of living organisms and their components in air, became a full-fledged scientific discipline in 1964. This was followed in 1974 by the founding of the International Association of Aerobiology (https://sites.google.com/site/aerobiologyinternational/). The initial focus of this group was the study and movement of pollen, but microbes and other life forms were soon added to the mix with a corresponding broadening of the organization's scope (Fig 1). The microbiologic quality of indoor air comes under the rubric of aerobiology (Fig 2). This workshop will focus only on indoor air as a vehicle for human pathogens.

Table 2
Workshop presentations in chronologic order

Presentation title	Speaker name, affiliation, and relevant experience	Subject matter to be covered
Welcome	John A. Mitchell: Wordsmith Scientific & Regulatory, Bozeman, MT	
Introduction, objectives, and expectation of outcome	Syed A. Sattar: Professor Emeritus of Microbiology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada His research focuses on the survival of human pathogenic microbes in the environment and how their environmental spread can be interrupted with physical and chemical agents. His expertise includes aerobiology of indoor air, where he has pioneered the development of several methods to study the airborne survival of viruses and bacteria and their inactivation.	Introduction of the topic with a set of definitions, clearly defined objectives, and expected outcome of the deliberations
Assessing microbial decontamination of indoor air, with particular focus on human pathogenic viruses	Caroline Duchaine: Professor, Université Laval's Research Center, Quebec Heart & Lung Institute, Ste.-Foy, QC, Canada Her research foci include aerobiology of animal and human pathogenic microbes, sampling of air for infectious aerosols, surrogates for human pathogenic viruses, and the application of molecular approaches to the study of bioaerosols.	Approaches to the study of pathogen survival and inactivation in indoor air, with particular emphasis on quantitative methods
Spread of infectious agents in the indoor environment	Yuguo Li: Professor, Mechanical Engineering, University of Hong Kong, Pokfulam, Hong Kong He studies the impact of the built environment and aerosolized droplet evaporation-dispersion of respiratory pathogens indoors, including engineering control of aerosolized infectious agents.	Impact of the built environment and aerosolized droplet evaporation-dispersion of respiratory pathogens indoors, including engineering control of aerosolized infectious agents
Generic aspects of airborne spread of human pathogens indoors and emerging air decontamination technologies	M. Khalid Ijaz: Research Fellow, RB, Montvale, NJ, and Adjunct Associate Professor of Biology, Medgar Evers College of The City University of New York (CUNY), New York, NY His research focuses on the spread of human pathogenic microbial agents via the environment, including air, and the mitigational role of hygiene, including air decontamination.	Approaches to the study of pathogen survival and inactivation in indoor air, with particular emphasis on quantitative methods
Mathematical modeling and simulation of bacterial distribution in an aerobiology chamber using computational flow dynamics	Bahram Zargar: Postdoctoral fellow at the Department of Biochemistry, Microbiology & Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada In addition to his expertise in aerobiology, his training and experience in mechanical engineering are major assets in experimental design, data analysis, and modeling of the behavior of pathogens in indoor air.	Mathematical models to predict the movement of pathogens indoors and the potential impact of air decontamination technologies
Aerobiology of the built environment—synergy between *Legionella* and fungi	Absar Alum: Assistant Professor of Research, Arizona State University, Tempe, AZ Expertise in the role of the environment in survival and spread of human pathogens.	Studies on interactions between different microbial species in biofilms in the built environment with a focus on legionellae
Knowledge gaps and future directions	Syed A. Sattar and M. Khalid Ijaz: Please see previously listed speaker descriptions.	Wrap-up with summary statements on the topics covered and a proposed agenda for research investigations, including recommendations for surrogate microbial agents
Panel discussion	All speakers	A set of preformulated questions will be presented for discussion
Vote of thanks	Joseph R. Rubino: Director of Research and Development, RB, Montvale, NJ	

Table 3
Glossary of common terms

Term	Definition	Comment
Aerosolization	Converting a liquid or powder into an aerosol; also called atomization and nebulization	Experimental aerobiology depends on equipment and techniques for safe and effective nebulization of the test microbe.
Aerobiology	Study of living organisms and their components in air	This umbrella term includes the study of indoor air quality.
Aerosols	Particles released into the air from a liquid or solid matrix	How long such particles remain suspended depends on the combined influence of the nature of the matrix, air turbulence, light, air temperature, and relative humidity.
Airborne spread	Spread of infections via inhalation of an infectious agent	Such spread requires that respirable particles carrying infectious agents remain suspended in air long enough to be inhaled by a potential host.
Allergens	Materials derived from plants (eg, pollen), fungi (eg, β-[1, 3]-glucan), bacteria or other biologic and nonbiologic sources capable of causing allergic reactions in a host	Whereas there are synthetic chemicals such as plastics capable of inducing allergic reactions, the emphasis here is on biologic materials only.
Biofilm	A slimy, multicellular layer of microbes on moist or submerged surfaces	This often is a mix of several types of microbes, including opportunistic pathogens.
Biological decay	Loss in viability of a given microbe by damage to ≥1 of its biologic functions	Airborne pathogens show different rates of biologic decay depending on their nature, prevailing environmental conditions, and fluid of their origin.
Chembioaction	A negative health outcome from the combined effect of a chemical and biologic agent	This phenomenon is especially relevant in aerobiology because a host is often exposed to potentially harmful biologic, chemical, or physical agents simultaneously or sequentially.
Droplet nuclei	Airborne particles derived from larger droplets after loss of water	Such droplets are crucial for the spread of infectious agents by air as their relatively small size (0.5-5.0 μm) allows for their stability in air while also permitting their retention on inhalation.
Indoor air quality	Quality of the air within buildings and other enclosures, with particular reference to the health and comfort of the occupants	The overall quality of indoor air is dependent on a mix of a variety of factors that may be site and time sensitive.
Infectious agent	A microbe capable of causing an infection	The capacity of a microbe to infect a given host depends not only on its biology but also on the general health status of the host and the portal of entry into the host.
Microbial pathogen	A microbe capable of causing localized or generalized damage to the host	Please see "Infectious agent."
Microbiome	The totality of microorganisms and their collective genetic material present in or on the human body or in another environment	A certain proportion of the microbes found in a microbiome may not be culturable but detected and identified via their genomes only.
Microbiota	A natural mix of bacteria, fungi, viruses, and protozoa in a host or an environmental niche	This term is now preferred over microflora.
Opportunistic pathogen	A microbial pathogen capable of infecting hosts whose natural defenses are compromised because of advanced age, immunosuppression, or other underlying causes	The number, variety, and health significance of such pathogens is on the rise in conjunction with the rising numbers of those debilitated by acquired or induced immunosuppression.
Pathogen (microbial)	Any microbe capable of causing damage to the host	Even an otherwise innocuous microbe can become pathogenic depending on the general resistance of the host or the microbe's entry into normally sterile areas of the body where it can become an opportunistic pathogen.
Perikairots	Environment-based opportunistic pathogens	Biofilm-based microbes such as legionellae and environmental mycobacteria can infect those debilitated because of age or underlying medical conditions.
Resident microbiota	A mix of microbes normally found in or on the host	Many members of the resident microbiota from skin and mucous surfaces are frequently found in indoor air.
Respirable particles	Particles small enough to access the alveolar space during normal breathing	Such particles may or may not contain viable microbes.
Surrogate microbe	A microbe that resembles ≥1 type of pathogens but is safer and easier to work with in the laboratory; also called a simulant	The use of such microbes is crucial in many aspects of microbiology, in general.
Tidal breathing	The body's automatic inhalation and exhalation process at rest	In addition to coughing and sneezing, tidal breathing can release infectious agents into the air.
Transient microbiota	Microbes temporarily acquired by a host during normal contact with the environment	

Indoor air quality

Exposure of humans to indoor air and its contents coincided with cave dwelling >200,000 years ago.[5] Sharing of the human habitat with domesticated animals, such as cattle, dogs, and pigs, facilitated the rise of zoonotic infections, including airborne infections (eg, measles).[6] Exposure to pathogens of humans and animals via the agency of indoor air continues to this day.

Although the focus here is on indoor air, indoor air is not entirely immune to what goes on outdoors. The air from outside an edifice affects the air indoors and vice versa. In fact, the use of fossil fuels for heating the indoors contributes directly and indirectly to overall climate change. An early consequence of energy conservation was sealed buildings and houses, which eventually gave rise to sick building or tight building syndrome as a result of the trapping of airborne pollutants and higher levels of moisture inside.[7]

Spread of airborne pathogens indoors

Humans and animals are the main contributors of microbe-laden particles indoors. In fact, individuals leave their own personal microbial footprint as a part of the indoor microbiome.[8] Aerosolization of microbes from biofilms and resuspension of dust are the other principal contributors to the microbial content of indoor air (Fig 3). Although the route by which airborne pathogens cause infections varies between microorganisms, improvements in the quality, quantity, and movement of indoor air can mitigate the airborne spread of many human pathogens by preventing pathogen

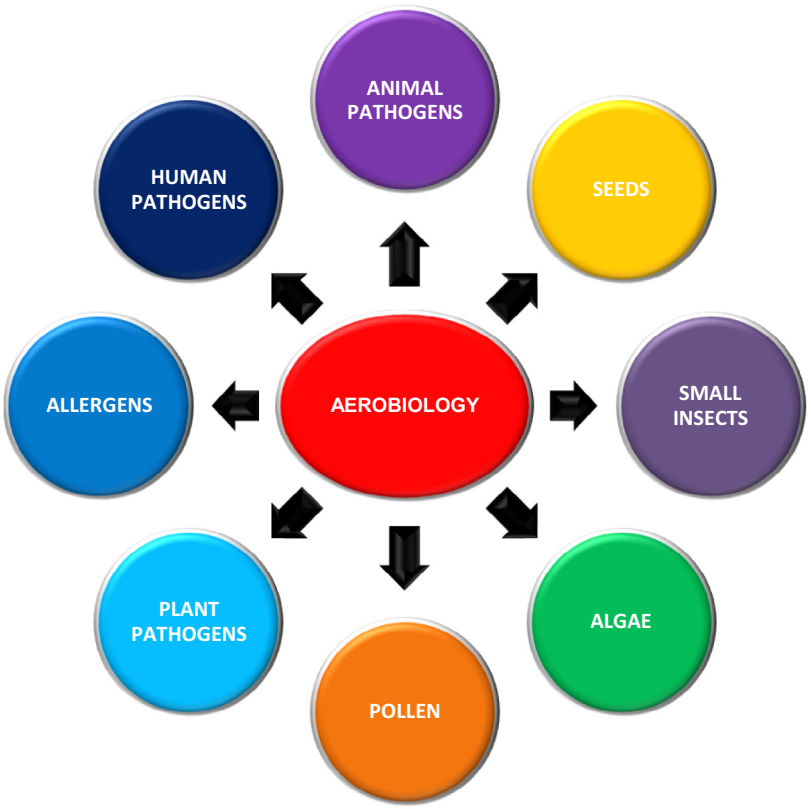

Fig 1. Components of aerobiology.

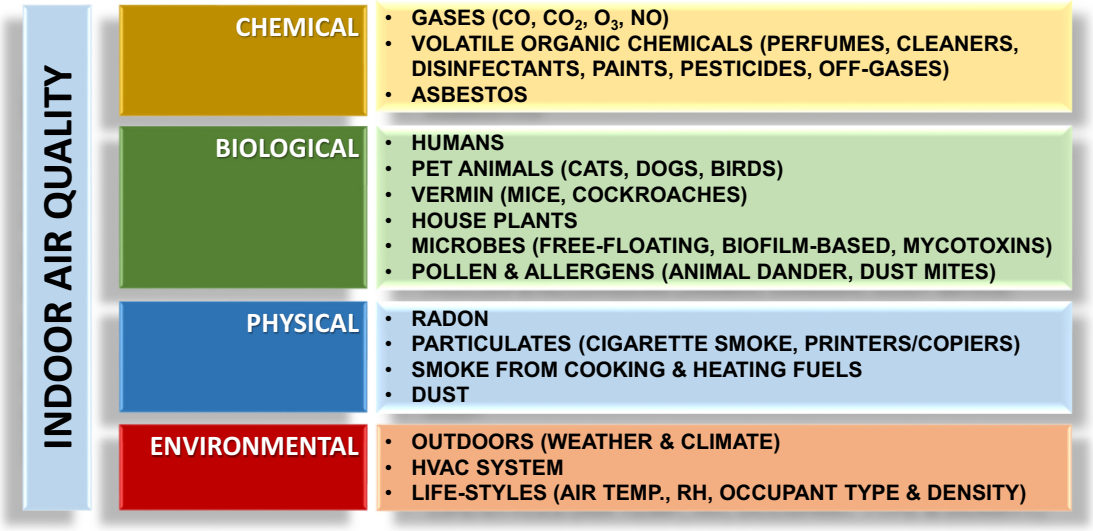

Fig 2. Factors affecting indoor air quality. *HVAC*, heating, ventilation, and air conditioning; *RH*, relative humidity.

inhalation and reducing the microbial load on environmental surfaces.

Indoor air is arguably the fastest and most highly efficient means of pathogen spread in a given setting. As depicted in Figure 2, indoor air is a complex and dynamic mixture of numerous components in a constant state of flux influenced by many factors both indoors and outdoors. The quality of indoor air represents the outcome of the unique mix of components in a given setting that, in themselves, change temporally.

One major challenge in preventing and controlling the airborne spread of infection is the presence of possibly multiple and mobile sources of pathogens at a given location and time. One or more infected or colonized persons or pets may contaminate the air in their immediate vicinity with exposure of those nearby without the air having reached any available means of pathogen decontamination.

Certain factors that influence indoor air quality may fall under the categories of chemical and physical. For example, smoke from

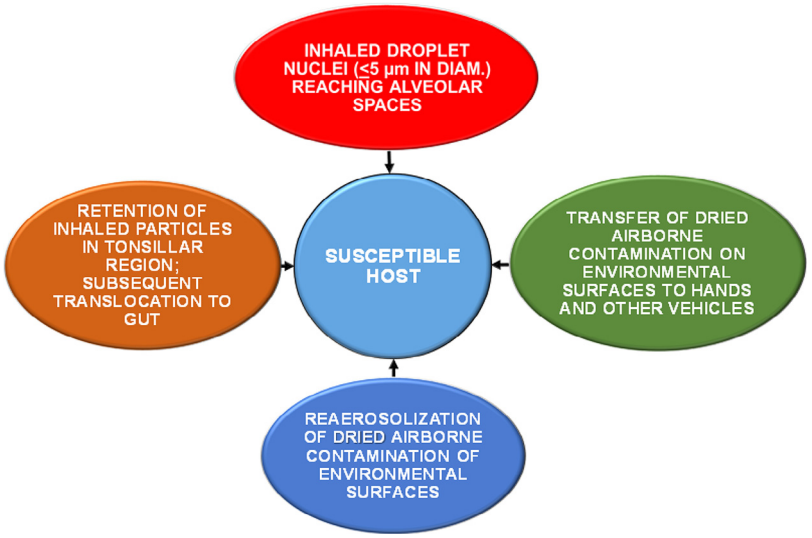

Fig 3. Airborne spread of human pathogens. *DIAM.*, diameter.

burning wood for cooking fuel is, of course, chemical in nature, but respirable particles in the smoke are the primary means of lung irritation and potential damage leading to cardiopulmonary syndromes, including lung cancer (http://www.who.int/mediacentre/factsheets/fs292/en/).

The study of indoor air quality received a major boost as a consequence of the severe acute respiratory syndrome outbreak in 2003[9] and the anthrax scares in the United States in 2011.[10] It also spawned much interest in the development, assessment, and application of technologies to decontaminate indoor air.

As shown in Figure 3, particles >10 μm in diameter entering the air may rapidly fall out of the air because of their mass under prevailing environmental conditions, particularly temperature and relative humidity, whereas smaller particles can not only remain airborne for extended periods but can also be transported readily indoors by air currents over considerable distances. Respirable particles fall in the range of 0.2-5.0 μm in diameter, whereas smaller particles are generally exhaled because of the aerodynamics of breathing. The actual site of retention of the inhaled particles depends on their nominal size. It is noteworthy here that persons with respiratory infections breathe out pathogen-laden particles during tidal breathing.[11]

A human adult at rest breathes in an average of 11,000 L of air per day.[12] In any given setting, one may choose not to drink the water or eat the food that is available but generally has little choice in breathing the same air as everyone else. This makes air an environmental equalizer—conferring on it the unique potential to parse out evenly whatever it may contain. Further, infectious agents entering indoor air mix rapidly with no perceptible color or smell.

Although the potential of air to spread respiratory pathogens is well recognized, its ability to transmit enteric pathogens is not as well appreciated. Airborne particles containing enteric pathogens may be retained in the tonsillar region and swallowed for relocation to the gastrointestinal tract with subsequent replication there.[13] Ijaz et al[14] have provided a comprehensive list of human pathogens known or suspected to spread via indoor air.

WHAT TO EXPECT FROM THE WORKSHOP

The following are the main topics to be covered during the workshop.

Generic test protocols for the study of airborne pathogens under field-relevant conditions

Despite the recognized significance of indoor air as a vehicle for human pathogens, there are major gaps in our understanding of how well these pathogens remain viable under different environmental conditions. Such information is crucial to assessing the potential of a given pathogen to spread by air. Construction of an aerobiology chamber (approximately 24 m^3) will be described, and data from use of the chamber to test airborne survival of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* will be presented.

Assessment of newer air decontamination technologies against pathogens

Many technologies claiming microbial decontamination of indoor air are on the market, but without proper validation of their claims. Information will be presented on ways to test such technologies using standardized protocols for registration and marketing purposes.

Selection of better surrogates to study airborne human pathogens

Because they may not be readily available and generally are unsafe and difficult to culture in the laboratory, it is rarely possible to use actual field strains of human pathogens in testing. This necessitates the use of surrogate microbes to generate data predictive of the behavior of pathogens. However, certain surrogates that are used commonly and recommended by regulatory agencies and standards-setting organizations alike are inherently unsuitable for experimental work in aerobiology. For example, *K pneumoniae*, frequently used as a surrogate for airborne gram-negative bacilli, does not survive aerosolization well because it is relatively fragile and unstable in air. Therefore, data generated with *K pneumoniae* likely will not be predictive of the behavior of actual human pathogens. This workshop will identify more suitable surrogates with supporting data.

Can microbial decontamination of indoor air reduce the risk for pathogen contamination of environmental surfaces?

Data will be presented to demonstrate that reductions in the levels of airborne microbes can indeed lead to corresponding

reductions in the microbial contamination of environmental surfaces in a given setting. The use of integrated models could help analyze outbreaks, evaluate the relative importance of hygiene and infection prevention and control for policymakers, and provide guidance in environmental design for greater occupant safety and comfort. These will be illustrated using some recent examples, including severe acute respiratory syndrome, influenza, and Middle East respiratory syndrome.

Update on the recovery and quantitation of viable microbes in indoor air

Quantitative recovery of viable microbes from air is vital in aerobiologic studies. An update will be given on available methods, including their strengths and limitations.

Mathematical modeling to help better design experimental chambers for work in aerobiology

Experimentation with airborne microbes is generally quite labor intensive and costly and requires special biosafety precautions. Mathematical models can assist greatly in optimizing aerobiology chamber design and in predicting the influence of furniture and other objects on the movement of microbes. Data will be presented with specific reference to a chamber that fully conforms to guidance from the U.S. Environmental Protection Agency.

Significance of biofilms as sources of pathogens in the built environment

Biofilms are not only common in the built environment, but they can be common sources of airborne pathogens. Such biofilms often contain several microbial species having complex interactions between them. This will be illustrated with the example of how fungi and legionellae coexist with potential risks to human health.

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Global Perspective Article

Airborne spread of infectious agents in the indoor environment

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Key Words:
Respiratory droplet
indoor air
coughing
droplet dispersion
infection control
environmental ventilation

Background: Since the 2003 severe acute respiratory syndrome epidemic, scientific exploration of infection control is no longer restricted to microbiologists or medical scientists. Many studies have reported on the release, transport, and exposure of expiratory droplets because of respiratory activities. This review focuses on the airborne spread of infectious agents from mucus to mucus in the indoor environment and their spread as governed by airflows in the respiratory system, around people, and in buildings at different transport stages.

Methods: We critically review the literature on the release of respiratory droplets, their transport and dispersion in the indoor environment, and the ultimate exposure of a susceptible host, as influenced by airflows.

Results: These droplets or droplet nuclei are transported by expired airflows, which are sometimes affected by the human body plume and use of a face mask, as well as room airflow. Room airflow is affected by human activities such as walking and door opening, and some droplets are eventually captured by a susceptible individual because of his or her inspired flows; such exposure can eventually lead to long-range spread of airborne pathogens. Direct exposure to the expired fine droplets or droplet nuclei results in short-range airborne transmission. Deposition of droplets and direct personal exposure to expired large droplets can lead to the fomite route and the droplet-borne route, respectively.

Conclusions: We have shown the opportunities for infection control at different stages of the spread. We propose that the short-range airborne route may be important in close contact, and its control may be achieved by face masks for the source patients and use of personalized ventilation. Our discussion of the effect of thermal stratification and expiratory delivery of droplets leads to the suggestion that displacement ventilation may not be applicable to hospital rooms where respiratory infection is a concern.

Since the 2003 severe acute respiratory syndrome epidemic, the 2009 H1N1 influenza pandemic, and the 2014 Middle East respiratory syndrome epidemic, scientific exploration of infection control is no longer restricted to microbiologists or medical scientists. Fluid mechanics has played a role in understanding the mechanism of transmission and in developing engineering interventions; for example, the studies of airflow dynamics by Yu et al[1] provided plausible evidence of airborne transmission of severe acute respiratory syndrome. Airborne spread of infectious agents is directly relevant to the airborne route, and indirectly to the droplet-borne and fomite routes. Breathing, talking, sneezing, and coughing are major sources of some respiratory pathogens. Up to 40,000 droplets are expelled at a velocity of 100 m/s during a sneeze,[2] and a cough can generate approximately 3,000 droplet nuclei.[3] We now understand to some degree where and how respiratory droplets are formed and the pathogen content in each size of droplet. Turbulence and coherent structures in the airflow, mostly invisible, transport respiratory droplets between people. For example, vortex structures in coughing probably carry particles over long distances.[4] Our body's thermal plumes can bring fine droplet nuclei upward, and vortices generated during door opening and wakes behind walking individuals can transport contaminated air out of an isolation room. Turbulence generated by supply air jets causes mixing and dilution of room air. Understanding these airflows is crucial to minimizing spread of infectious agents and infection transmission.

Here, we review the release of respiratory droplets, their transport and dispersion in the indoor environment, and the ultimate

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exposure of a susceptible host, as influenced by airflows. Microbial survival in the environment is beyond the scope of this article.

RELEASE, TRANSPORT, AND EXPOSURE

Release of droplets from mucus to mouth

If we understand the mechanism of where and how respiratory droplets are generated, we may have opportunities to suppress them at the source. Knowing the number and size of respiratory droplets is also crucial.

First, the content of infectious agents expelled by an infected person depends largely on the location within the respiratory tract where the droplets originate. Pathogenic microorganisms tend to be found in certain locations, particularly the tonsils and the larynx, and seldom at the front of the mouth.[5] Three droplet size distribution modes have been proposed: the bronchiolar fluid film burst mode, containing droplets produced during normal breathing ($d \leq 1$ μm); the laryngeal mode, most active during voicing and coughing ($d \geq 1$ μm); and the oral cavity mode, active during speech and coughing, producing droplets ≥100 μm.[6,7] The oral cavity is among the sources of expiratory droplets, especially larger ones[5,8,9]; the droplet formation mechanism in the oral cavity is shown in Figure 1. Large droplets from the trachea produced during coughing might not be released into the environment because they readily deposit within the head airways. Johnson et al[6] found that droplets ≥20 μm only originate from the oral cavity. Droplets generated during breathing may originate from both the upper and lower airways, but the latter seems to make the major contribution because of the film rupture mechanism.[12,13]

Second, 2 major mechanisms exist for droplet formation in the respiratory tract (Fig 1). One is the instability caused by the shear stress on the mucus-air interface that leads to the avalanche of mucus and droplet formation. The biphasic airway lining fluid has an overall thickness ranging from 5-100 μm. A critical air speed is required to initiate the instability, which varies according to mucus thickness, its viscoelastic properties, and surface tension at the mucus-air interface. Coughing is one mechanism for mucus clearance, during which air speed as high as 200 m/s can be attained[14] and interfacial shearing is peaked within the trachea.[15] This mechanism has traditionally been considered an exhalation process during coughing and sneezing; however, it was found to be also plausible around the first bifurcations during inhalation.[15,16] Recent studies include the effect of viscoelastic properties and surface tension on the onset of instabilities (eg, Vasudevan and Lange[17,18]) and the effect on the size distribution and volume concentration of bioaerosols produced.[19]

During normal tidal breathing, however, the shear force provided by the respiration airflow is not sufficient to induce instabilities. The mechanism for droplet formation during normal breathing relates to the reopening of collapsed terminal airways at the beginning of inspiration. Almstrand et al[20] examined the production of exhaled particles after varying degrees of airway closure. Concentrations of exhaled particles showed a 2- to 18-fold increase after exhalations to residual volume, compared with exhalations where no airway closure was shown. Malashenko et al[21] defined a critical capillary number ($Ca = \mu U/\sigma$, where μ is the dynamic viscosity of the liquid, U is the axial speed of the air-liquid meniscus propagation, and σ is the surface tension between the lining fluid and the air) above which droplets may be formed during normal breathing. In addition, experiments simulating the film droplet formation process showed that small fluid films generate droplets as efficiently as large films, and droplets may well be generated from films with diameters <1 mm (ie, the diameter of terminal bronchioles).[22]

Third, the reported number and size of released droplets vary significantly. In terms of the total mass of saliva, 1.1-6.7 mg of saliva were collected on a mask during a single cough, and 18.7 mg were collected while counting from 1-100.[9,23] There were 1-320 droplets per liter of exhaled air found for breathing, 24-23,600 found for coughing, and 4-600 found for speaking.[7,23-31]

Several factors account for the significant inconsistencies between the existing data (eg, individual differences, imperfect measurement techniques, and effect of evaporation). Humidity level is crucial in the measurement of droplet and droplet nuclei sizes.[29] Various measurement methods have been used, such as microscopic

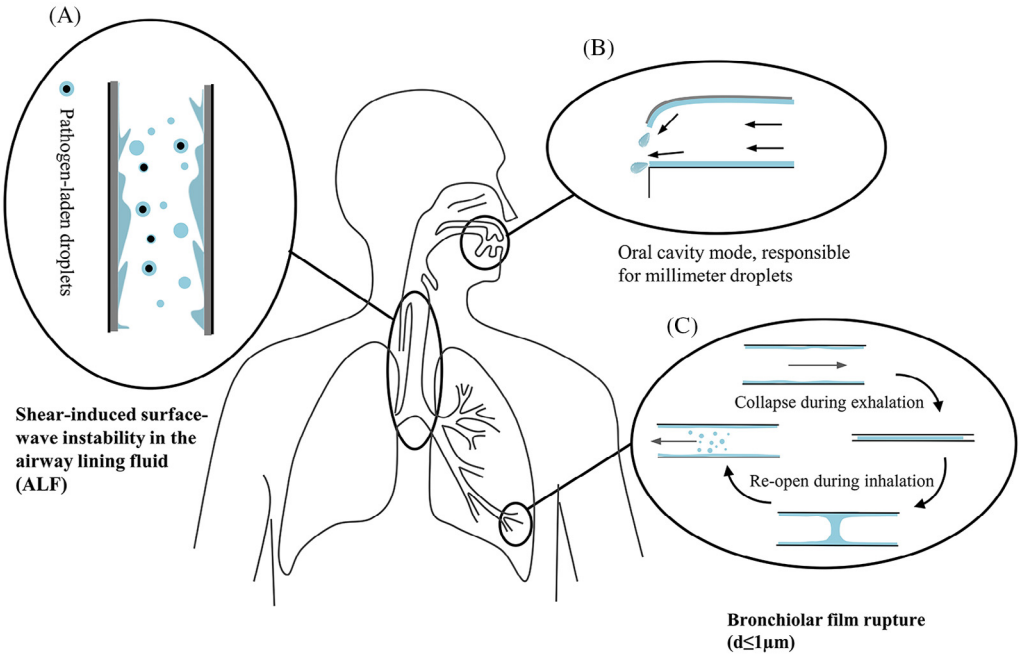

Fig 1. Schematic diagram revealing the origin and generation mechanism of respiratory droplets. (A) Instability of the airway lining fluid[10], (B) oral cavity model (drawn based on the atomization mechanism described in Morawska[5]), and (C) film rupture.[11]

observations,[8] optical particle counting,[25] aerodynamic particle counting and scanning mobility particle sizing,[29] interferometric Mie imaging,[30] and laser aerosol particle spectrometry.[31] Lindsley et al[31] found that individuals infected with influenza virus produce a significantly greater volume of aerosol during clinical illness compared with during the asymptomatic stage ($P = .0143$). This enhancement in aerosol generation during illness may play an important role in influenza virus transmission.

Finally, we are interested in the quantity of pathogens in each size category of aerosols. The size of viruses varies from 0.02-0.3 μm, and the size of bacteria varies from 0.5-10 μm in their naked form. It is anticipated that small viral pathogens travel readily within the lungs and between individuals and their environment in small droplet nuclei. The influenza virus RNA detected by quantitative polymerase chain reaction in human exhaled breath suggests that it may be contained in fine particles generated during tidal breathing.[32,33] Lindsley et al[34] measured the content of influenza virus in aerosol particles from human coughs. Thirty-five percent of the influenza RNA detected were contained in particles >4 μm in aerodynamic diameter, whereas 23% were contained in particles 1-4 μm and 42% were in particles <1 μm, suggesting that much of the viral RNA is contained within particles in the respirable size range. *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has been reported in small droplet nuclei, and patients have generated bacteria-laden aerosols in a diameter range of 0.65-4.7 μm during coughing.[35]

Spread of droplets from the mouth and nose to the indoor environment

When the expiratory flow is weak during the full respiratory cycle, the body plume also plays a role. Weak expiratory flows (eg, those blocked by the use of a face mask) may be captured by the body plume (Fig 2). Expiratory flows are also where most droplet nuclei are formed under typical room conditions. Exhaled flow rate over time may be represented as a sinusoidal function for breathing, a constant for talking, and a combination of gamma probability distribution functions for coughing.[36,37] The peak velocities of coughing and breathing can be 6-22 m/s (>10 m/s on average) and 1-5 m/s, respectively.[30,36,38] The differences in reported initial velocities are mainly attributed to different measurement techniques and individual variability.

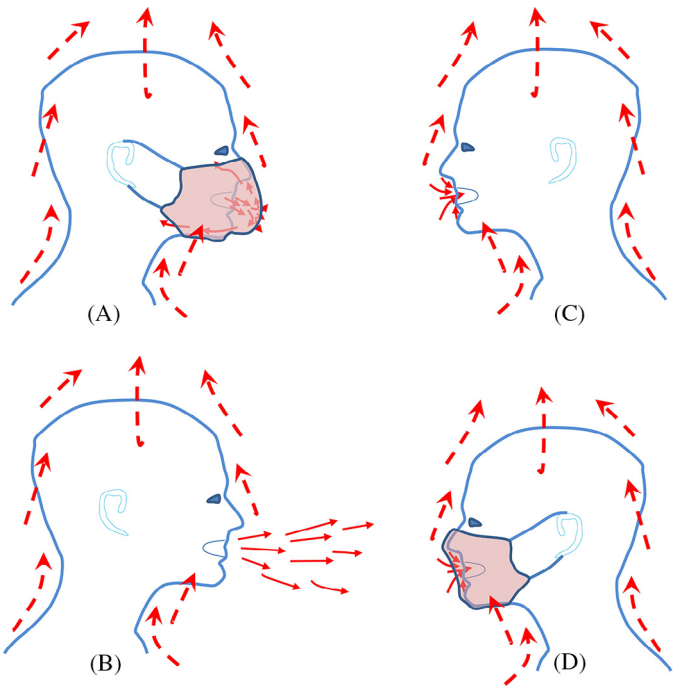

Fig 2. Escaped microbial aerosols of an infected individual with a mask (A) and without a mask (B) as affected by the body plume and inhalation of the airborne infectious agent(s) of a nearby individual without a mask (C) and with a mask (D).

Among all respiratory activities, coughing has probably been studied the most. The Schlieren technique using human volunteers reveals the turbulent cough jet with a leading vortex,[39] and the cough has properties similar to a starting jet or puff.[40] The vortex ring structure produced during coughing is also important in particle dispersion.[4] An aerosol-laden jet, led by a characteristic vortex, can penetrate an impressive distance into the surrounding ambient air before finally mixing out.[41] Xie et al[42] found that expired droplets can travel 1.5-2 m. The presence of turbulence greatly enhances droplet spread.[43]

Water droplets with sizes on the order of 1 μm evaporate within a few milliseconds, water droplets of 10 μm survive for up to a few tenths of a second, and large droplets of 100 μm can survive for almost a minute. The transient process from a droplet to a droplet nucleus can be ignored when studying the movement of small droplets,[44] whereas evaporation barely affects large droplets because they deposit soon after release. However, medium-sized droplets (eg, 50 μm in diameter) are most sensitive to humidity.[43] Deposition occurs either by gravitational sedimentation, turbulent eddy impaction, or diffusional deposition. Large droplets were first defined as droplets >100 μm by Wells.[45] In respiratory exhalation flows, the largest droplets that would completely evaporate before traveling 2 m are between 60 and 100 μm.[42] These large droplets are carried >6 m by exhaled air at a velocity of 50 m/s (sneezing), >2 m at a velocity of 10 m/s (coughing), and <1 m at a velocity of 1 m/s (breathing). Particles of diameters 1-3 μm remained suspended almost indefinitely, whereas those with a diameter of 10 μm took 17 minutes to fall 3 m to the floor, those with a diameter of 20 μm took 4 minutes, and those with a diameter of 100 μm took 10 seconds.[46] For small droplets, gravitational sedimentation is negligible; however, turbulent eddy impact or diffusional deposition may occur. Small droplets are subject to the ambient velocity field, and their evaporation clearly assists in transporting them a significant distance.[47]

Spread of droplets in the indoor environment

The transport of expiratory droplets can be considered in terms of 2 stages, with the primary being the expiratory flow, followed by secondary dispersion via room airflow. The airflow in buildings is typically designed to be <0.25 m/s on average for thermal comfort. Typical airflows are turbulent, and they are affected by many parameters, such as air distribution systems,[48-50] room furniture setup, body thermal plumes,[51-53] and human conversational behavior and activities.[54-56] These influences are illustrated in Figure 3. However, droplet size seems to be the most important factor affecting dispersion and deposition.[5] Size of droplets affects their dispersion and deposition on surfaces and the survival of microorganisms within the droplets. Physical characteristics of the indoor environment, such as temperature and relative humidity and design of the ventilation system, are also important.[5] The survival of pathogens inside the droplets is likewise subject to various environmental conditions and has been reviewed by Tang.[58]

Here we specifically discuss the effect of human walking and body plumes. The airflow behind a human body is complex because of the complexity of body shape and movement. Generally, there are 2 distinct wake regions: an unsteady bluff-body wake behind the torso and a region of unsteady vortex shedding behind the legs, which is dominated by a jet of air formed between the legs.[54] A

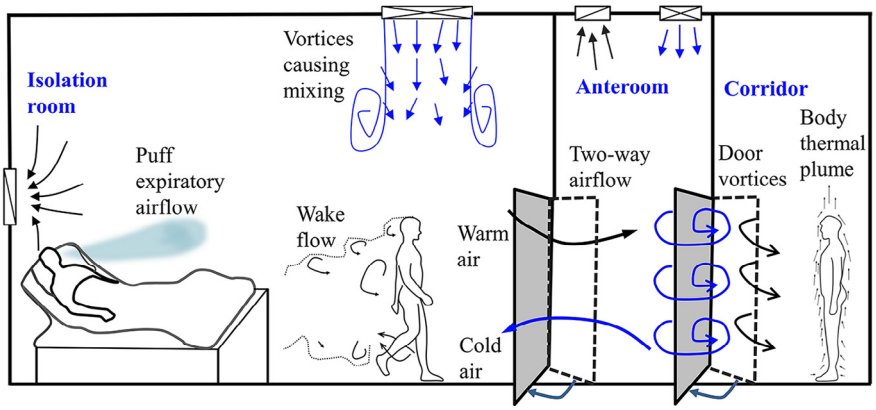

Fig 3. Droplet transport in an isolation room by expired airflow, thermal plume[51], door vortices (adapted with permission from Elsever[57]), human walking[54], 2-way buoyancy airflow, and ventilation airflow.

significant downwash that occurs behind the body has the effect of laterally spreading the lower portions of the wake.[59,60]

Using large eddy simulation, Choi and Edwards[55,61] found that backward transport (opposite to the direction of walking) can also occur because of downwash effects and tip vortex formation. Wake-induced transport of material in the direction of the walking motion continues because of inertial effects, even after the person stops. When the walking effect is combined with hinged door opening, the latter is the dominant transport mechanism, and human-induced wake motion enhances compartment-to-compartment transport. In addition, when isolation room air has a temperature different from that of the corridor, the 2-way airflow effect at the openings plays an important role in aerosol dispersal.[62]

The body thermal plume starts from the feet as a laminar flow and grows in both its velocity and thickness upward along the human body. It is important in the individual microenvironment and inhalation.[51-53] The plume becomes fully turbulent at the middle chest level. It reaches a maximum velocity (0.2-0.3 m/s) approximately 0.5 m above the head. The thickness of the plume can reach 15 cm in the breathing zone, so airflow from the lower part of the human body is drawn into the mouth during inhalation, which makes up approximately two-thirds of total inhaled air. The total air flux in the plume is in the 20-35 L/s range.[63] The rising thermal plume entrains and transports pollutants when the pollution source is on the floor, leading to a higher concentration in the microenvironment, particularly in the breathing zone of the standing or seated person, more so than in the ambient environment.[64,65] When the cough of a source patient penetrates the area around another person's lower body, the thermal plume can bring the fine droplet nuclei upward.

The thermal plume also can act as an air curtain to protect the person from the penetration of airflow expired by other people.[66] In displacement ventilation, the reduction in plume buoyancy caused by stratification is substantial.[63] In downward ventilation, the thermal plume can be preserved at head height if it meets the downward air at 0.25 m/s,[50] which compromises the transport dominated by the thermal plume. Many factors influence the thermal plume (eg, gestures, clothing insulation, the blocking effect of a table, movement of people).[51,67,68] When walking at a speed of >0.2 m/s, the effect of the thermal plume would give way to the human aerodynamic wake.[54] It is worth mentioning that plumes induced by other heat sources also contribute to pollutant transport. In the smallpox outbreak in Meschede, Germany,[69] a radiator in the index patient's room introduced an upward plume flow because of a partially open window, resulting in the spread of smallpox.

Exposure of susceptible hosts to respiratory droplets

A susceptible host can be close to a patient (eg, during conversation) or at a distance from a patient (eg, sharing the same classroom) but sitting sufficiently far away.

For 2 people in close contact, exposure can be caused by the direct spray route during which large droplets are deposited directly on the mucous membranes of the susceptible host (large droplet route) or by direct inhalation of fine droplets or droplet nuclei (airborne route). The latter is referred to as the short-range airborne route because exposure occurs when the 2 individuals are in close contact. For both the large droplet route and the short-range airborne route, expired droplets from the infected person can penetrate the thermal plume of the susceptible host, reaching the mucus or inhalation zone of the susceptible individual (Fig 2).[66]

When the susceptible individual is sufficiently far from an infected individual, direct inhalation of the contaminated room air is referred to as the airborne route. The infection risk of the susceptible host caused by inhaled droplets depends on the quantity of pathogen he or she carries and on the site at which the droplets deposit within the respiratory tract. Inhaled particles can deposit in different regions of the respiratory tract (eg, head airway region, tracheobronchial region, pulmonary region). Deposition mechanisms include inertial impaction (limited to large particles), settling (most important in small airways), Brownian motion of submicrometer particles, and interception.[70] Recent studies on airflow and particle transport in the human respiratory tract were reviewed by Kleinstreuer and Zhang.[71] Airflows are complex in the nasal cavities and oral airways; particles deposit largely at stagnation points, disrupting axial particle motion. According to the International Commission on Radiological Protection model[72] for adults engaged in light work, total deposition is dominated by deposition in the head airways of particles >1 μm; the number of particles >10 μm that can penetrate the head airways is negligible.

We define 3 major routes of droplet exposure (Fig 4): the direct spray route, the long-range airborne route, and the fomite route, which is not discussed here. The direct spray route can be divided into 2 subroutes in terms of size and destination of the expiratory droplets and droplet nuclei: the short-range airborne route (<10 μm) and the droplet-borne route (>10 μm). This is basically in line with the definitions from the U.S. Centers for Disease Control and Prevention[73]; however, we distinguish the short-range and long-range airborne routes.

The definition of the transmission route of a specific pathogen also must account for its virulence and infectious dose, and differ-

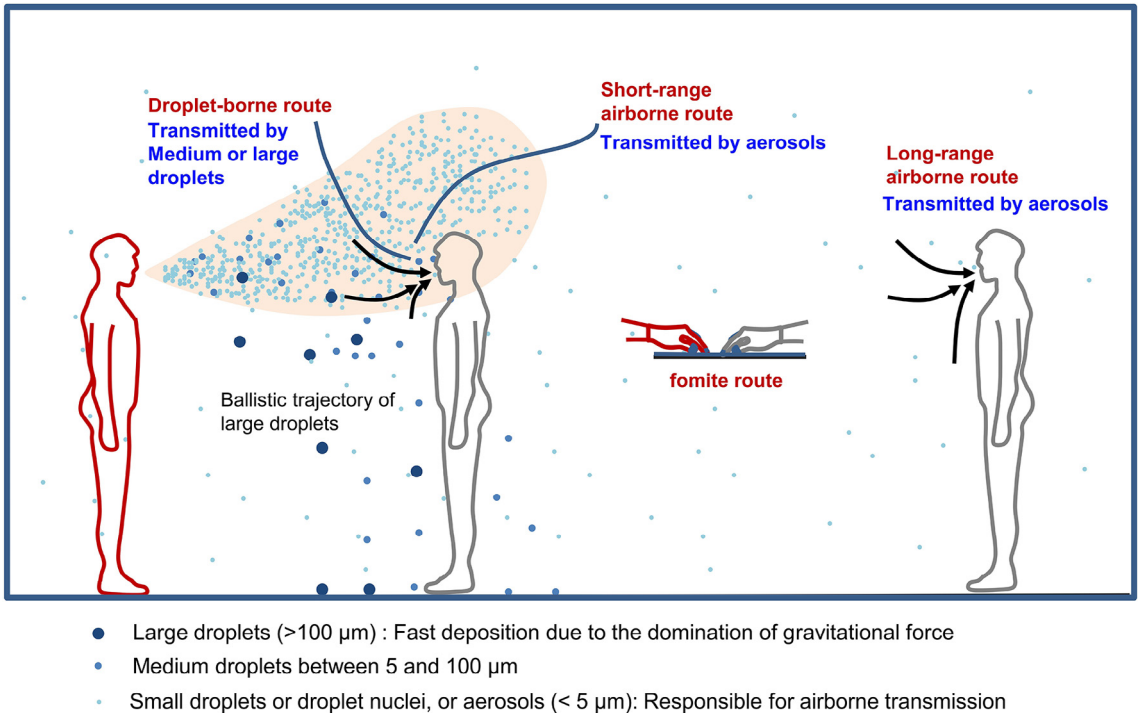

Fig 4. Illustration of different transmission routes. Small droplets (<5 μm), sometimes called aerosols, are responsible for the short-range airborne route, long-range airborne route, and indirect contact route; large droplets are responsible for the direct spray route and indirect contact route.

ent modes are not mutually exclusive. The infectious dose of a pathogen is the number of microorganisms required to cause an infection. Data from research performed on biological warfare agents suggest that both bacteria and viruses can produce disease with as few as 1-100 infectious units (eg, brucellosis: 10-100 infectious units, Q fever: 1-10 infectious units, tularemia: 10-50 bacterial cells, smallpox: 10-100 infectious units, viral hemorrhagic fevers: 1-10 viral particles).[74] There remains considerable controversy over the relative importance of the alternative modes of transmission of influenza virus. Brankston et al[74] concluded in a review that natural influenza virus transmission in humans generally occurs over short distances, rather than over long distances, whereas Tellier[75,76] concluded that aerosol transmission occurs at appreciable rates. Weber and Stilianakis[77] found that contact, large droplet, and small droplet (aerosol) transmission are all potentially important modes of transmission for influenza virus. Our purpose here is not to make conclusions about the relative importance of each route but to comment on the impact of airflows on the spread of infectious agents.

RELEVANCE TO INFECTION CONTROL

Respiratory infection could be reduced or eliminated by interruptions in 3 phases: release of pathogen at the source, transport of pathogen by air or by surface touch, and protection of the susceptible person.

Prevention of droplet release at origin by saline inhalation

There are 2 ways of altering mucus properties.[17,18] The first is to lower the mucus viscosity and increase elasticity and surface tension for total suppression, and the second is to enlarge droplet size by decreasing the elasticity and surface tension and increasing the viscosity. The latter approach is preferred because the droplets generated would be smaller and more dangerous if full suppression was not achieved. Edwards et al[26] found that delivering approximately 1 g of isotonic saline orally via nebulized aerosols (droplets 5.6 μm in diameter) reduced the total amount of expired aerosols (among super-producing individuals) by approximately 72% over a 6-hour period. In vitro tests using a simulated cough machine indicated that a mucus mimetic nebulized with saline produces a larger droplet size after the forced convection of air over its surface than when air is forced over the mucus mimetic alone (ie, without saline nebulization). In a subsequent study, Clark et al[78] report that delivering isotonic saline aerosols (5.6-μm droplets) into the endotracheal tube of anesthetized bull calves showed a dose-responsive effect on exhaled bioaerosols; 6 minutes of treatment resulted in a decrease of up to 50% of exhaled aerosols for at least 120 minutes, compared with the pretreatment case. Inhaling safe surface-active materials, such as isotonic saline, to suppress exhaled bioaerosols was reviewed and recommended for controlling airborne transmission[79]; however, more studies are required to clearly elucidate the potential of this new approach.

Use of masks for infected individuals and for susceptible individuals

Two reviews[80,81] highlight the limited evidence base supporting the efficacy of face masks in reducing influenza virus transmission. They suggested that surgical masks may reduce infectiousness, rather than protect against infection, especially when airborne transmission is important. Influenza viruses (with sizes in the 80- to 120-nm range) and other viruses of similar size are capable of penetrating the mask in either direction. The N95 respirators are efficient in removing very fine droplet nuclei, but face masks are not. However, face masks, if worn by an infected person, can suppress the expired jets (Fig 2A) and reduce the close contact transmission via both the droplet-borne and short-range airborne routes.

Environmental ventilation for the long-range airborne route

A multidisciplinary systematic review[49] suggested that ventilation rate and airflow patterns contribute directly to the airborne spread of infectious agents; however, the minimum ventilation rate for effective airborne transmission control is unknown at present. The current minimum requirement is 12 air changes per hour for negative-pressure airborne isolation rooms.[82,83] Natural ventilation may offer a low-cost alternative.[83,84] The current negative-pressure isolation rooms with a ceiling supply and bottom return system are recommended, but gaseous and fine particles were found to be removed more efficiently by ceiling-level exhausts, and large particles were removed mainly by deposition, rather than by ventilation.[85] Displacement ventilation has been recommended as a more energy-efficient approach in nonhospital settings. However, in the case of the isolation room, the stable thermal stratification zone may cause the lock-up phenomenon to occur[86] if the exhaled pollutant is not caught by the thermal plume penetrating into the upper zone, resulting in a longer residence time of pollutants.[87] Displacement ventilation can create what might be referred to as inversion clouds in rooms. Because deposition is the main mechanism for removing large droplets,[85] floor cleaning in hospitals is absolutely necessary.

Personalized ventilation for the short-range airborne route

This may be a less well-known technology in the infection control community. Its principle is based on detectable jets of air with a high momentum directed at a person's face.[88,89] It may not be effective when the mobility of the subject is considered. An air supply pillow was suggested for hospital use.[90] The personalized ventilation (PV) system can be supplemented with a general ventilation system in the room. Experiments with PV, together with vertical ventilation from ceiling-mounted terminals, show increased efficiency of personal protection by a factor of up to 35.[90] A combination of PV and the personalized exhaust method was suggested.[91]

CONCLUSIONS

By reviewing the airborne spread of infectious agents from mucus to mucus in the indoor environment, we have shown the opportunities for infection control at different stages of the spread. We propose that the short-range airborne route may be important in close contact, and its control may be achieved by face masks for the source patients and the use of PV. Our discussion of the effect of thermal stratification and expiratory delivery of droplets leads to the suggestion that displacement ventilation may not be applicable to hospital rooms where respiratory infection is a concern. The saline inhalation method was discussed after a discussion of the mechanisms of droplet formation and origin.

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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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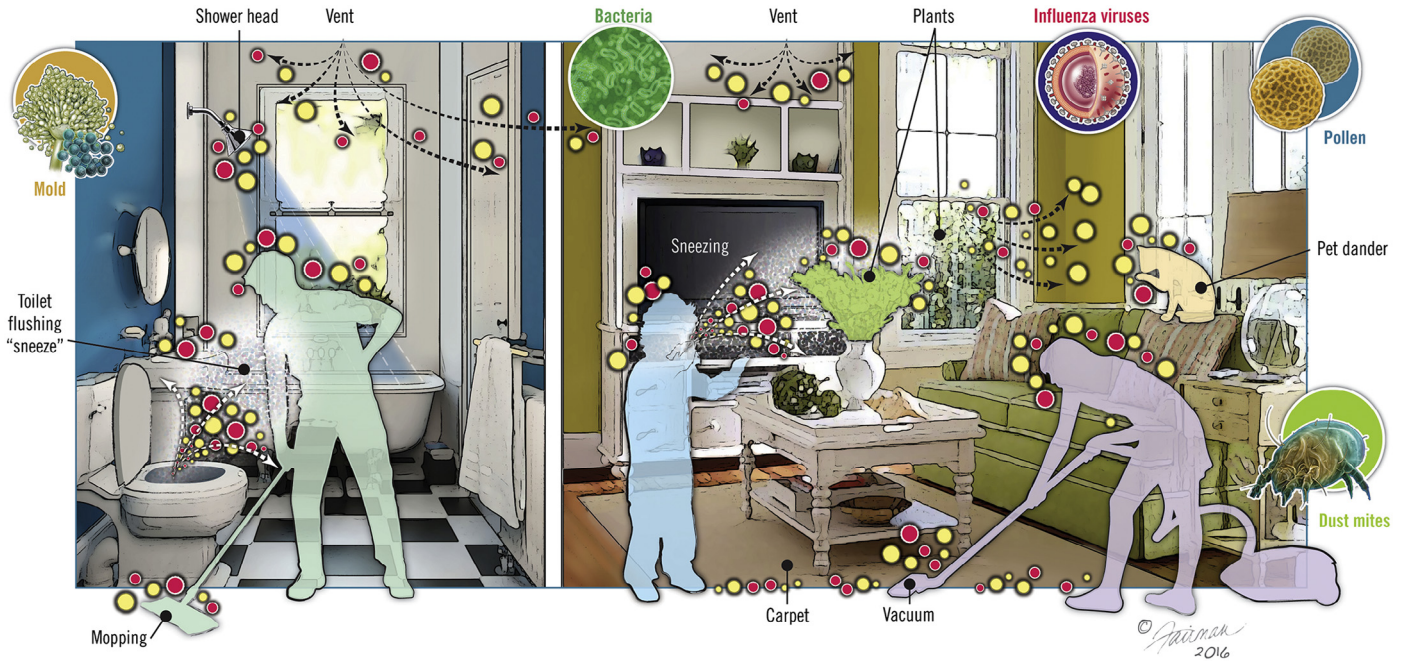

Fig 1. Sources of airborne pathogens indoors and potential for environmental surface contamination. These sources may include humans; pets; plants; plumbing systems, such as operational toilets and shower heads; heating, ventilation, vacuuming, mopping, and air-conditioning systems; resuspension of settled 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]

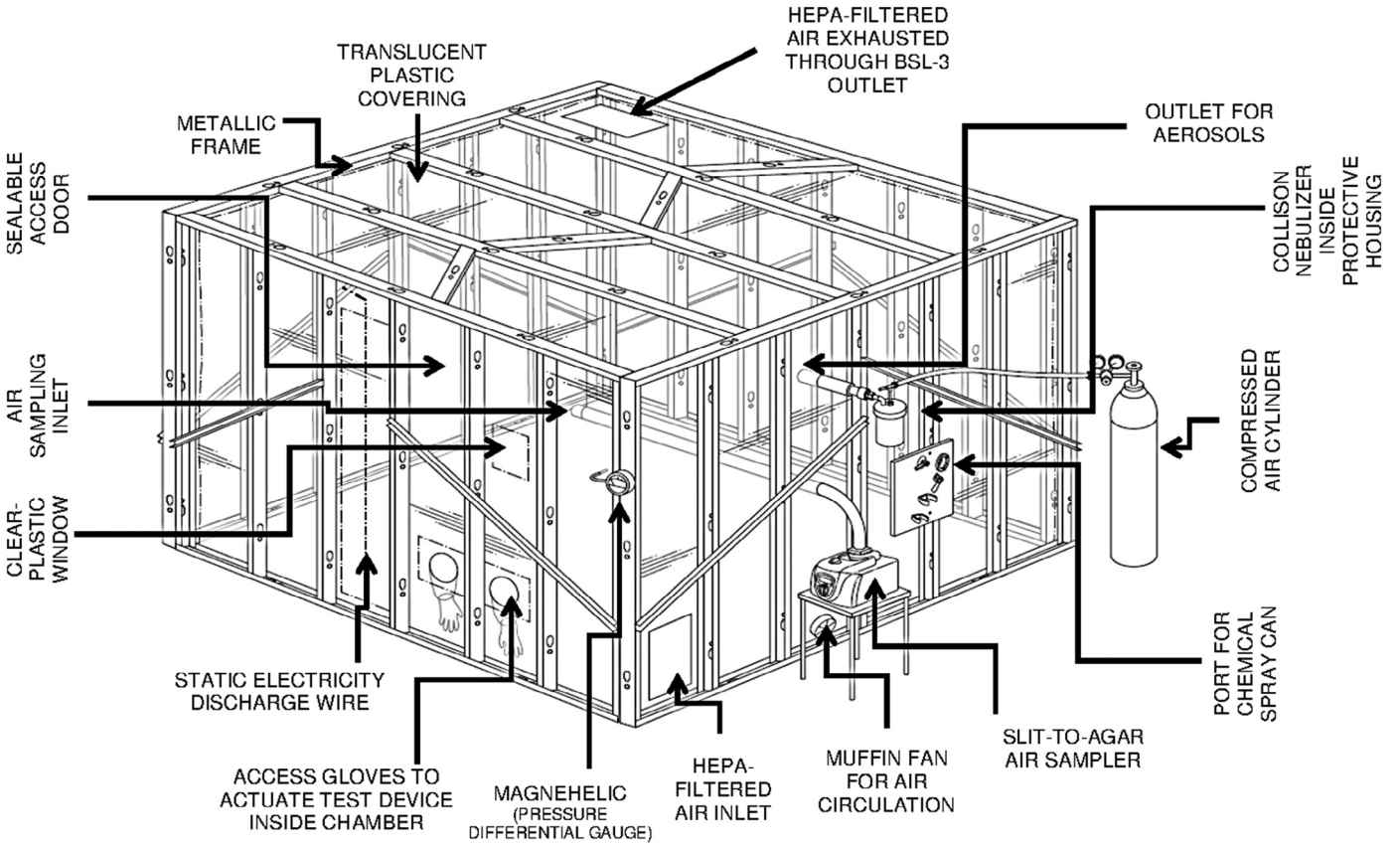

Fig 2. Aerobiology chamber with essential components (length × width × height: 320.0 × 360.6 × 211.0 cm—24.3 m^3 [860 ft^3]). Reprinted with permission from Elsevier.[73]

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-

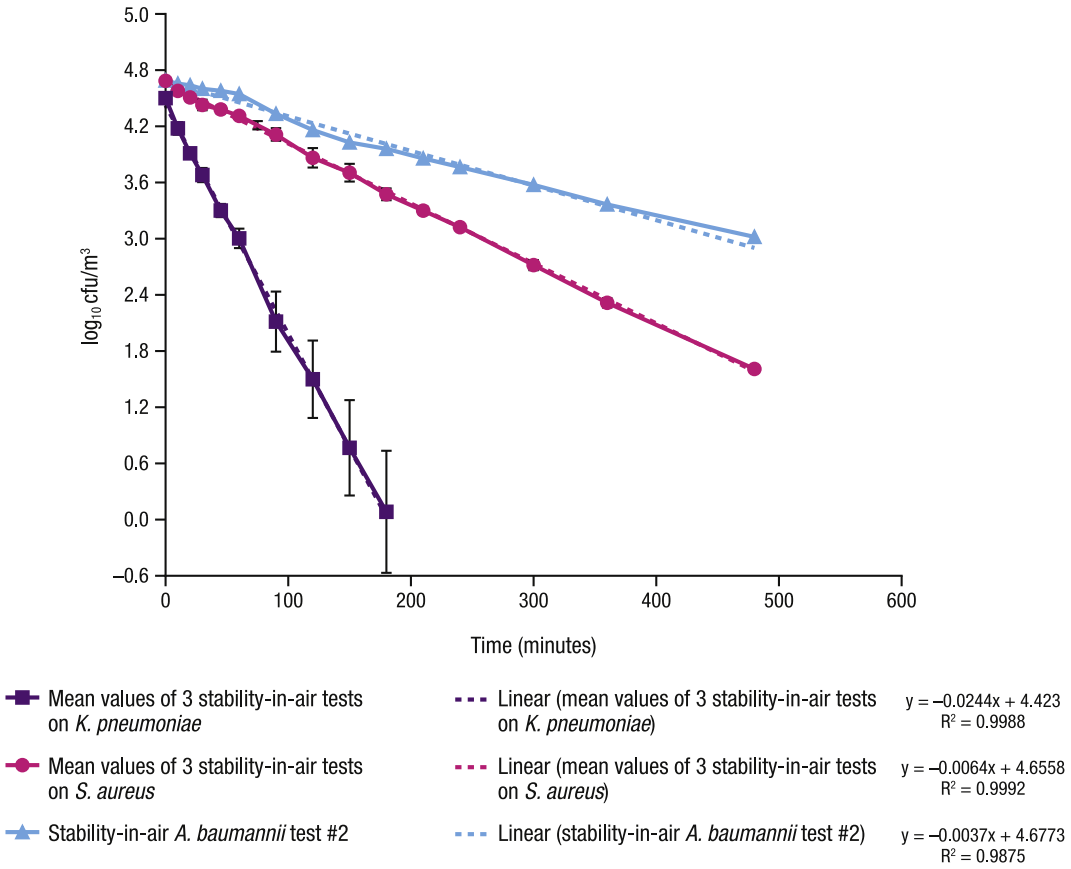

Fig 3. Comparative rates of biologic decay of aerosolized *A. baumannii*, *S. aureus*, and *K. pneumoniae* held within the aerobiology chamber. *A. baumannii*, *Acinetobacter baumannii*; *cfu*, colony forming units; *K. pneumoniae*, *Klebsiella pneumoniae*; *S. aureus*, *Staphylococcus aureus*.

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

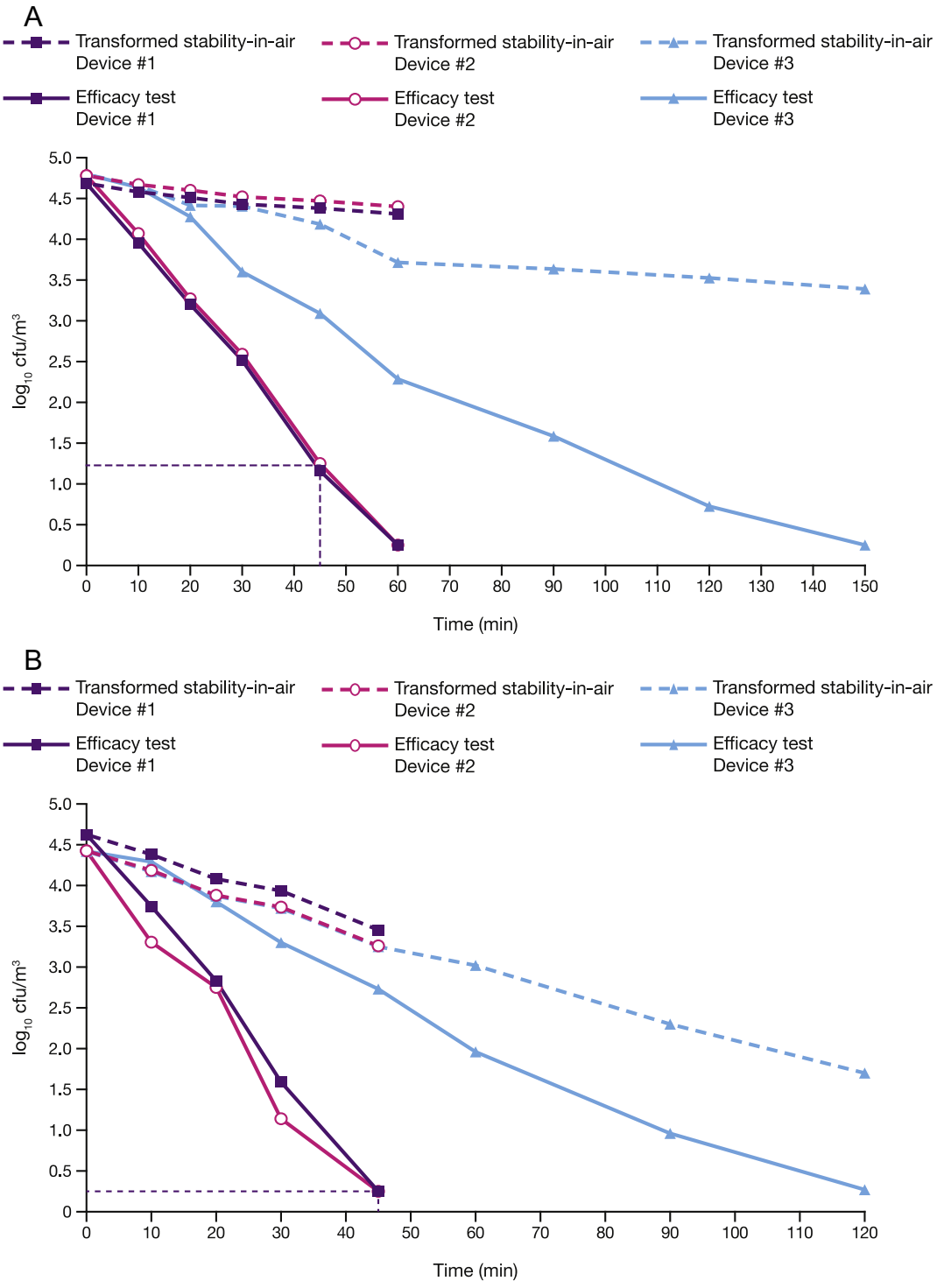

Fig 4. (A) Comparative inactivation rates of airborne *Staphylococcus aureus* during the operation of 3 indoor air decontamination devices. *cfu*, colony forming units. Reprinted with permission from Elsevier.[73] (B) Comparative inactivation rates of airborne *Klebsiella pneumoniae* during the operation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

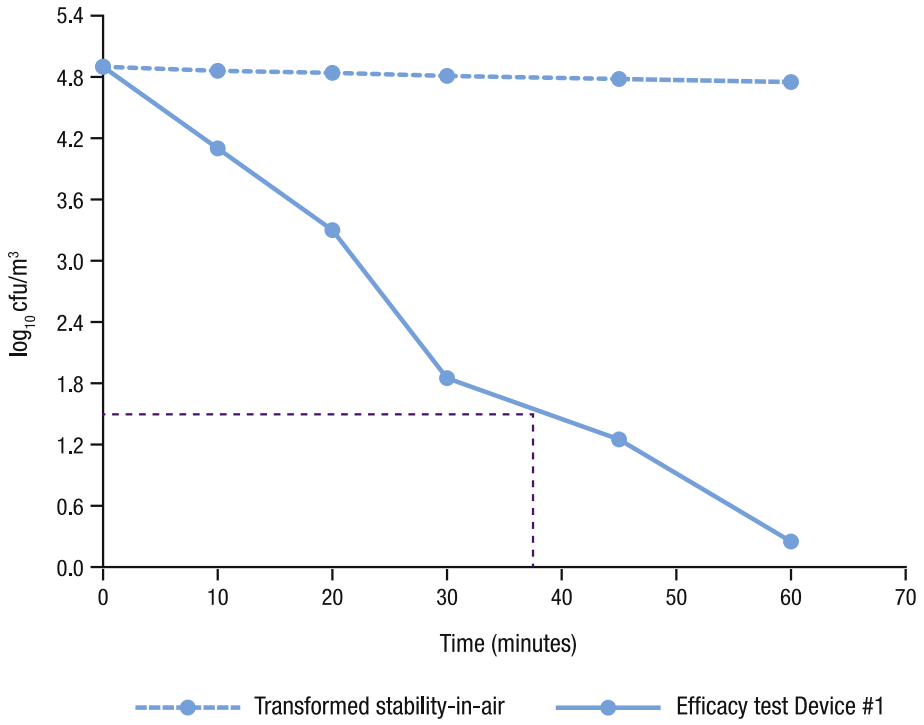

Fig 5. Inactivation of aerosolized *Acinetobacter baumannii* during operation of an indoor air decontamination device (device 1). *cfu*, colony forming units.

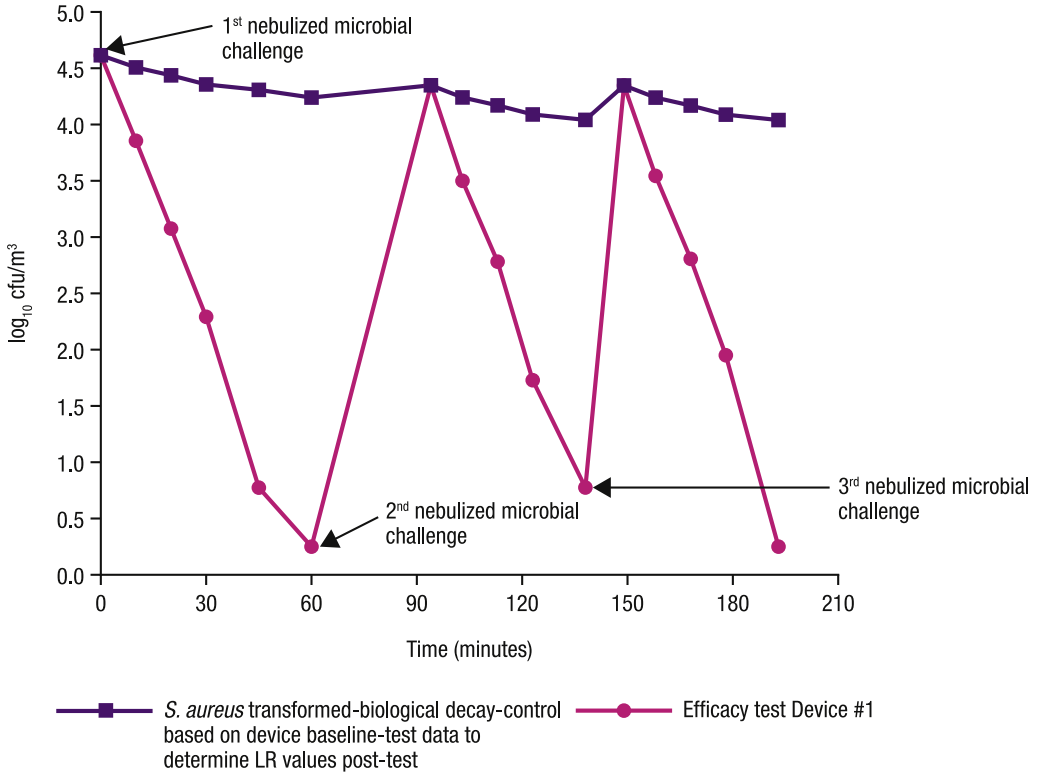

Fig 6. Repeated microbial challenge with aerosolized *S. aureus* during operation of device 1. *cfu*, colony forming units; *LR*, log10 reduction; *S. aureus*, *Staphylococcus aureus*.

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

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Transmission of bacterial, fungal, and viral pathogens is of primary importance in public and occupational health and infection control. Although several standardized protocols have been proposed to target microbes on fomites through surface decontamination, use of microbicidal agents, and cleaning processes, only limited guidance is available on microbial decontamination of indoor air to reduce the risk of pathogen transmission between individuals. This article reviews the salient aspects of airborne transmission of infectious agents, exposure assessment, in vitro assessment of microbicidal agents, and processes for air decontamination for infection prevention and control. Laboratory-scale testing (eg, rotating chambers, wind tunnels) and promising field-scale methodologies to decontaminate indoor air are also presented. The potential of bacteriophages as potential surrogates for the study of airborne human pathogenic viruses is also discussed.

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.[1] 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,[2] certain types of pathogens are obligated to spread by air only; pulmonary tuberculosis is a good example of this.[3] 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.[4]

For some airborne infectious agents, the respiratory system may not be the ultimate target. For example, epidemiologic evidence[5,6] 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.[7] The pandemic potential of human influenza viruses is related to their ability to spread by air.[8,9] In light of this evidence, safe and effective decontamination of indoor air would be an important adjunct to infection prevention and control.[10]

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 2,000 μm.[11-14] 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.[15]

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.[14]

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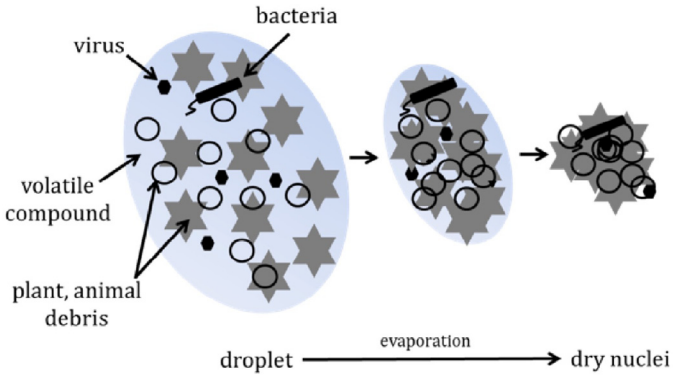


Fig 1. Droplet nuclei formation.

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.[16] Similar findings were obtained with other respiratory viruses: cytomegalovirus,[17] respiratory syncytial virus,[18] rhinovirus,[19] and the coronavirus responsible for the severe acute respiratory syndrome.[20]

EXPOSURE ASSESSMENT: FROM SAMPLING TO ANALYSIS

Indoor air often contains a varied and variable blend of microbes,[21] 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,[22] 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,[23] 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 approaches cannot readily distinguish between viable and nonviable microbes, therefore compromising their value in risk assessment and epidemiologic studies.

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.[24] 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.[25] 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.[26] 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.[27] 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.[28] Phage MS2 has been the most broadly used surrogate in aerosol studies and is used mostly in biodefense to predict the fate and transport of biothreat agents.[29] 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.[30]

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.[16] 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.

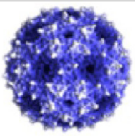
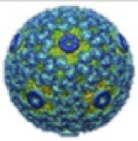
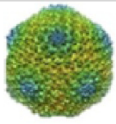
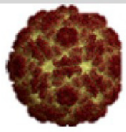
Although it would be highly desirable to assess any indoor air decontamination technology against all major types of airborne microbial threats before its adoption, time and cost constraints and the unavailability of suitable test protocols essentially preclude such an approach. Furthermore, the in-field 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.[28] Aerosols can remain airborne for prolonged periods in rotating chambers[31] 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.[32] 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[28] (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.[23] Mechanical ventilation is more

Table 1
Characteristics of the 4 phage models developed in previous studies

Phages	MS2	Phi6	PR772	PhiX174
Family	*Leviviridae*	*Cystoviridae*	*Tectiviradae*	*Microviridae*
Capsid	•One structural protein •Icosaedric •27 nm	•Icosaedric •86 nm	•Double capsid •Icosaedric •53 nm	•Icosaedric •25 nm
Envelope	no	yes	no	no
Genome	•Single stranded •Linear RNA •Non segmented •3569 nucleotides	Double stranded •Linear RNA •3 segments •13 385 base pairs	•Double stranded •Linear DNA •Non segmented •14 492 base pairs	•Single stranded •Circular DNA •Non segmented •5386 nucleotides
Host	*E. coli*	*Pseudomonas syringae*	*E.coli*	*E.coli*
Incubation temperature	37°C	25°C	37°C	37°C
Similar eukaryotic viruses	*Picornaviridae* *Caliciviridae*	*Reoviridae* *Orthomyxoviridae* *Paramyxoviridae* *Retroviridae*	*Adenoviridae*	*Circoviridae*
Images				

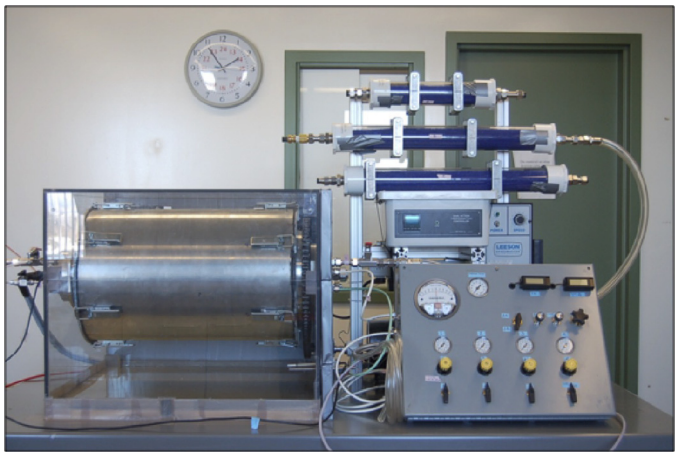


Fig 2. Rotating chamber for the study of aging bioaerosols and effectiveness of air decontamination approaches.

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.[33] RH also affects the viability of viruses.[34] 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 instable 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

ozone in these systems requires concentrations >5 ppm, concentrations at which ozone represents a risk for occupants.[39] 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.[23] Inactivation of microbial agents in indoor air has been addressed using photocatalysis as a function of the oxidizing power of ultraviolet radiations A (UVA)-irradiated semiconductors.[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.[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.[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.[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.[44] In hospitals, highly resistant pathogenic microbes, such as *Clostridium difficile* spores, are known to be present in the air.[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).[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,[49,50] and efficacy of bacterial and fungal aerosol capture has been studied as well.[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.[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*.[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.[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.[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.[57,58]

Other methods

Plasma discharge has been tested for the microbiologic decontamination of air and has been shown to be efficient against filamentous fungi.[59,60] 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.[61] Photocatalysis effectively destroys a wide range of gram-negative and gram-positive bacteria and fungi, algae, protozoa, and viruses.[62] 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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Major Article

Mathematical modeling and simulation of bacterial distribution in an aerobiology chamber using computational fluid dynamics

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Key Words:
Airborne spread of infectious agents
Distribution of particles in indoor air
Sampling air for infectious agents
Predictive modeling of bacterial distribution in an aerobiology chamber

Background: Computer-aided design and draft, along with computer-aided engineering software, are used widely in different fields to create, modify, analyze, and optimize designs.
Methods: We used computer-aided design and draft software to create a 3-dimensional model of an aerobiology chamber built in accordance with the specifications of the 2012 guideline from the Environmental Protection Agency for studies on survival and inactivation of microbial pathogens in indoor air. The model was used to optimize the chamber's airflow design and the distribution of aerosolized bacteria inside it.
Results: The findings led to the identification of an appropriate fan and its location inside the chamber for uniform distribution of microbes introduced into the air, suitability of air sample collection from the center of the chamber alone as representative of its bacterial content, and determination of the influence of room furnishings on airflow patterns inside the chamber.
Conclusions: The incorporation of this modeling study's findings could further improve the design of the chamber and the predictive value of the experimental data using it. Further, it could make data generation faster and more economical by eliminating the need for collecting air samples from multiple sites in the chamber.

INTRODUCTION

Prediction of particle transport in turbulent flow is essential in different fields, such as dispersion of passive or reactive particles in turbulent media and in studying air pollution.[1] For example, we are exposed to airborne particulates in workplaces, homes, and other indoor settings.[2] The fate and deposition of such particulates indoors have substantial implications for human and animal health, clean rooms, and air decontamination.[3-5] Therefore, a good understanding of the particle-laden turbulent flow is important in addressing indoor air quality issues and in controlling particle dispersion.

Mitigating the spread of microbial contaminants by indoor air is an essential design consideration for homes, biomedical and health care facilities, and other public settings. Once airborne, the movement of microbes is difficult to control because they may become rapidly dispersed by air movement or adhere to other surfaces for travel with them.[6,7] Ventilation, either natural or mechanical, can provide adequate air exchanges to reduce the risk for airborne microbial spread; however, mechanical ventilation, particularly with conditioning, can be expensive.[8] According to the *Guidelines for Design and Construction of Hospital and Health Care Facilities*,[9] 6-15 air changes per hour are needed to maintain a healthful environment while reducing exposure to harmful chemicals and microbes. This

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requires ventilation system engineers to understand microbial behavior in air to design more efficient and economical means of treating and supplying indoor air.[10]

In general, particles with a mass median aerodynamic diameter of 10 μm or less can remain airborne.[11] Memarzadeh and Xu[12] emphasized the importance of particle size in the airborne transmission of infections by transport of pathogen-laden particles to the mucosal surface of a susceptible host.[12]

Available information shows that ventilation systems can influence the spread of airborne pathogens indoors,[13,14] airflow patterns may contribute directly to such spread,[15] and airflow rates can influence the transport and removal of human expiratory droplets.[5,16-18] Assessing the risk of transmission of infections via air is more difficult than predicting reductions in concentrations of harmful gases with ventilation. Also, and unlike inhaled gases, it may take only a few infectious units of a given pathogen to infect a susceptible host, which, in turn, can amplify the level of the pathogen many-fold for further dissemination.

Increasing the air exchange rate alone is often inadequate for reducing the risk of spread of airborne infections everywhere within a given room. For optimal safety, the entire ventilation system should be analyzed to determine the likely path of pathogen-laden particulates within the occupied zones and the required corrective action.[19]

The 2 major approaches to study of the dispersion of particles in indoor air are physical modeling and numerical simulation with computational fluid dynamics (CFD). Empirical data are useful for CFD validation of air and movement of particulates in indoor environments and health care facilities. CFD modeling is also much more economical to perform than full-scale experimentation with actual pathogens or their surrogates.[20] Thus, with the ready availability and greater sophistication of CFD, it is increasingly being applied to predict room air movement in various types of health care settings.[21] However, this approach has not been adequately applied to other types of indoor settings and validated with experimental data[22]; when applied to predict airflow patterns in buildings, it was a flexible alternative to physical models.[22-24]

This study applies CFD to optimize and validate the performance of an aerobiology chamber that was designed based on Environmental Protection Agency guidelines.[25] The best location, angle, and speed of a muffin fan for producing uniform bacterial distribution were determined. The number of air sampling sites required for characterizing the distribution of the nebulized bacteria in the chamber was investigated. The stabilization time required to produce a uniform distribution of the bacteria was determined, and the effect of furniture on bacterial distribution also was studied.

METHODS

The dimensions of the studied aerobiology chamber were 320 cm × 360 cm × 210 cm.[26] The chamber was designed based on Environmental Protection Agency guidelines[25] and then used to study bacteria survival in air (Fig 1).[26] A 6-jet nebulizer was used to aerosolize bacterial suspensions into the chamber through a pipe with a 3.8-cm diameter. The air was sampled from the center of the chamber using a slit-to-agar machine via a 5.0-cm pipe. A muffin fan (Nidec Alpha V, TA300, Model A31022-20, P/N: 933314 3.0-inch/7.62-cm diameter; output 30 CFM; Nidec Corp., Braintree, MA) placed on the floor of the chamber directly beneath the nebulizer inlet pipe was actuated from the outside for continuous operation during nebulization and testing to ensure uniform distribution of the aerosolized particles and/or any treatment introduced. The procedure of the experiment was as follows:

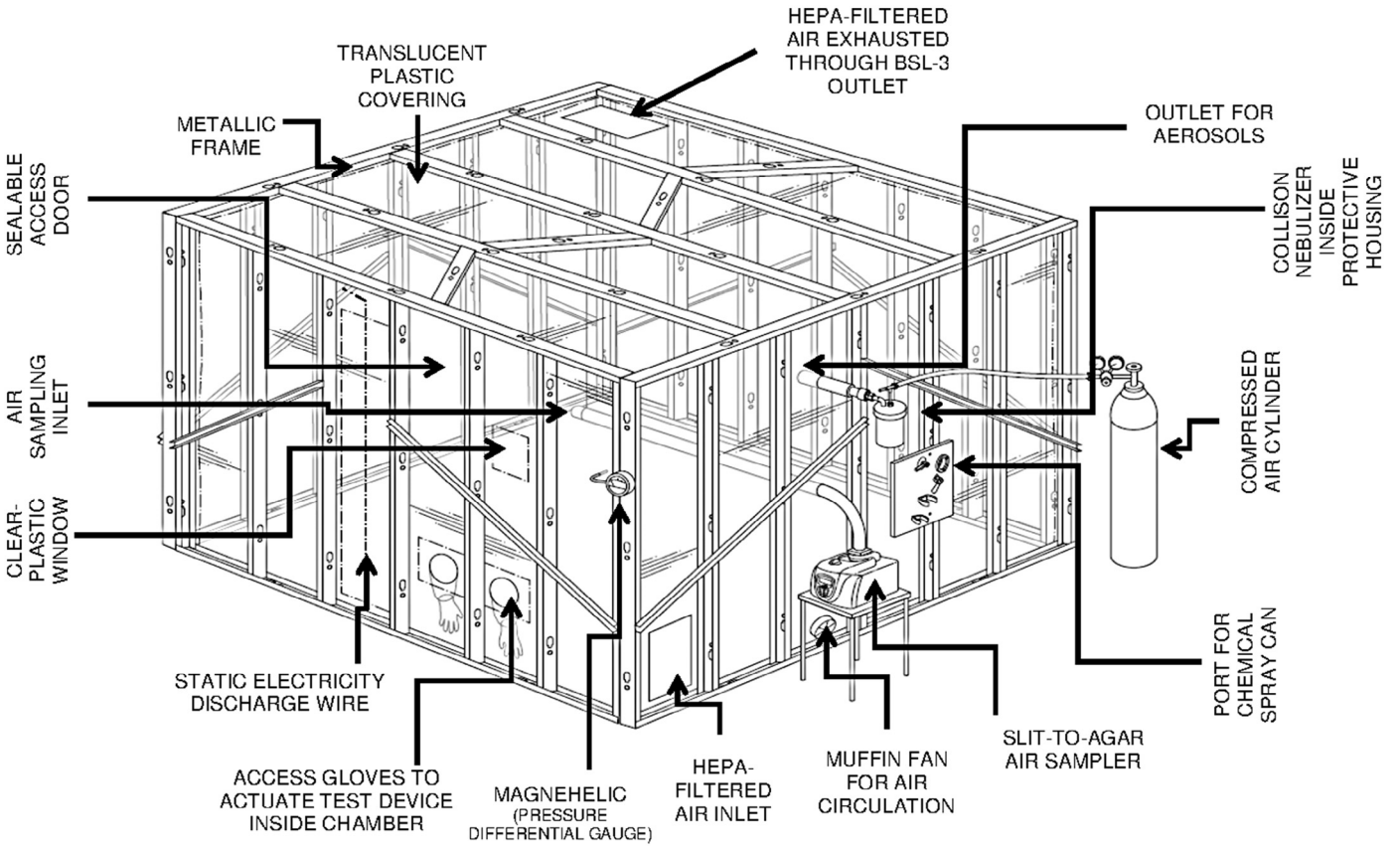


Fig 1. Aerobiology chamber designed based on Environmental Protection Agency guidelines.[25] Reprinted with permission.

- The fan was activated at least 300 seconds before the experiment to circulate the air inside the chamber;
- The test bacterial suspension was nebulized into the chamber for 10 minutes using a 6-jet collison nebulizer; and
- Before sampling, the air in the chamber was allowed to circulate for 300 seconds following the nebulization process.

MATHEMATICAL MODELING, MATERIALS, AND NUMERIC METHODOLOGIES

Theoretical background

The Eulerian-Lagrangian approach was implemented directly using the discrete phase model. In this approach, the fluid phase is treated as a continuum material by solving the time-averaged Navier-Stokes equations, and the dispersed phase is solved by tracking a large number of particles through the calculated flow field.[27] The governing equations are itemized in the following sections.

Governing equations for the continuous phase

The continuous gas-flow phase is governed by the following equations for unsteady compressible flow:[4]

- Continuity equation:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{u}) = 0 \tag{1}$$

- Momentum equation:

$$\frac{D(\rho \vec{u})}{Dt} = -\vec{\nabla} p + \nabla \cdot \tau \tag{2}$$

- Energy equation:

$$\frac{\partial (\rho E)}{\partial x} + \nabla \cdot (\vec{u}(\rho E + p)) = \nabla \cdot (k_{eff} \nabla T + (\vec{\tau}_{eff} \cdot \vec{u})) \tag{3}$$

Where ρ, t, u, r, p, τ, E, T, k_{eff}, and τ_{eff} are fluid density, time, fluid phase velocity, thermodynamic pressure, stress tensor, energy, temperature, effective conductivity, and effective stress tensor, respectively.

One of the most common turbulence models, the k-ε Realizable turbulence model, was used for turbulence modeling. The turbulence kinetic energy, k, and its rate of dissipation, ε, are obtained from the following transport equations:[27,28]

$$\frac{\partial}{\partial t}(\rho k) + \frac{\partial}{\partial x_i}(\rho k u_j) = \frac{\partial}{\partial x_i}\left[\left(\mu + \frac{\mu_t}{\sigma_k}\right)\frac{\partial k}{\partial x_j}\right] + G_k + G_b - \rho\varepsilon - Y_M + S_k \tag{4}$$

$$\frac{\partial}{\partial t}(\rho \varepsilon) + \frac{\partial}{\partial x_j}(\rho \varepsilon u_j) = \frac{\partial}{\partial x_j}\left[\left(\mu + \frac{\mu_t}{\sigma_\varepsilon}\right)\frac{\partial \varepsilon}{\partial x_j}\right] + \rho C_1 S_\varepsilon - \rho C_2 \frac{\varepsilon^2}{k + \sqrt{\upsilon \varepsilon}} + C_{1\varepsilon}\frac{\varepsilon}{k} C_{3\varepsilon} G_b + S_\varepsilon \tag{5}$$

Where G_k and G_b represent the generation of k due to the mean velocity gradients and buoyancy, respectively; Y_M represents the contribution of the fluctuating dilation in compressible turbulence to the overall dissipation rate; σ_k and σ_ε are the turbulent Prandtl numbers for k and ε, respectively; and S_k and S_ε are user-defined source terms for k and ε, respectively.

The turbulent (or eddy) viscosity, μ_t, is computed by combining k and ε as follows:[27,28]

$$\mu_t = \rho C_\mu \frac{k^2}{\varepsilon} \tag{6}$$

The model constants $C_{1\varepsilon}$, $C_{2\varepsilon}$, C_μ, σ_k, and σ_ε have the following values: $C_{1\varepsilon} = 1.44$, $C_{2\varepsilon} = 1.92$, $C_\mu = 0.09$, $\sigma_k = 1.0$, and $\sigma_\varepsilon = 1.3$.[27,28]

The rotating reference frame was applied only in the rotational region by assuming that the region was in a quasisteady state. This method does not explicitly generate model rotation; instead, it generates a constant grid flux in the appropriate conservation equations by automatically adding the source terms with respect to the Coriolis force and centrifugal force, which are calculated with equation 7 based on the properties of the reference frame.[27] Although this method underestimates the weak effect, it is appropriate for the flow, which is most likely to be influenced by time-averaged properties.[27] A significant amount of simulation time can be saved with this method, when compared with simulating the axial flow fan's rotation in a transient state.[27]

$$F_r = \rho \omega \times v \tag{7}$$

Where F_r is the body force term due to fan rotation (kg/m^2/s^2), ρ is the air density (kg/m^3), ω is the rotational speed (rad/s), and v is the linear velocity (m/s).

Governing equations for the discrete phase

The trajectory of the discrete phase is determined by integrating the force balance on the particle, which equates the particle inertia with forces acting on the particle, and can be written as:[27]

$$\frac{du_p}{dt} = F_D(u - u_p) + \frac{g_x(\rho_p - \rho)}{\rho_p} + F_x \tag{8}$$

Where u, u_p, g_x, ρ_p, ρ, and F_x are the fluid phase velocity, particle velocity, gravitational acceleration, particle density, fluid density, and an additional acceleration (force per unit particle mass), respectively. The drag force per unit particle mass (F_D) is equal to:

$$F_D = \frac{18\mu}{\rho_P d_p^2}\frac{C_D Re}{24} \tag{9}$$

$$Re \equiv \frac{\rho d_p |u_p - u|}{\mu} \tag{10}$$

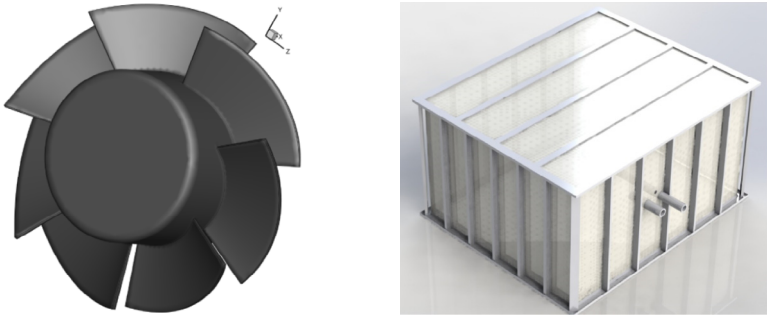
Where μ, d_p, C_D, and Re are the molecular viscosity of the fluid, particle diameter, drag coefficient, and Reynolds number, respectively. The location of each particle, x, is tracked with the following equation:

$$\frac{dx}{dt} = u_p \tag{11}$$

The air velocity, u, in equation 8 is composed of the time-averaged component, $\bar{u}$, and the instantaneous or fluctuating velocity component, $u'(t)$:[4,27]

$$u = \bar{u} + u'(t) \tag{12}$$

The u component is computed using the Reynolds-averaged Navier-Stokes equations with the k-ε Realizable turbulence model. The u′(t) component is computed using a stochastic approach, such as the discrete random walk model or eddy lifetime model.[4] Its value prevails during the lifetime of the turbulent eddy influencing the particle and is assumed to obey the Gaussian probability distribution.[4] Using the discrete random walk model to calculate u′(t), the particle turbulent dispersion is correlated to the flow k:[4,27]



(a) Axial flow fan model (b) Computational domain (aerobiology chamber) model

Fig 2. Three-dimensional model of the axial flow fan and computational domain.

$$u'(t) = \zeta\sqrt{\frac{2k}{3}} \tag{13}$$

Where the variable ζ is a Gaussian random number.

CFD procedure

Generally, flow simulations in CFD take place in 3 main stages. The first step is preprocessing, which includes geometric modeling, production of computational domain, and grid generation. The second is the processing step or flow solution with CFD. In the final step, called postprocessing, the results are displayed.

Geometric modeling

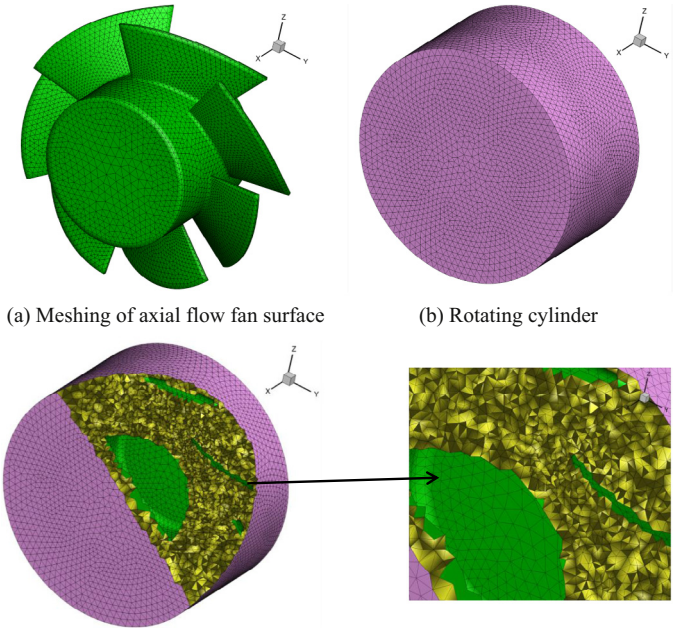
The geometry of the aerobiology chamber consists of several components, such as axial flow fan, fan housing, air sampler inlet pipe, outlet pipe for aerosol sampling, and aerobiology chamber walls. Each of these geometries is modeled separately, and eventually, with superposition of the modeled geometries, the final complex geometry is generated. The computer-aided design and draft model of the flow region is built based on the computer-aided design and draft model of the aerobiology chamber.

The muffin fan, which is an axial flow fan, presents the most complex geometry in the system. The axial flow fan is a tube-axial device with 7 forward-swept blades. The dimension of the fan housing is 80 mm × 80 mm × 40 mm. The tip diameter of fan blades, hub-to-tip ratio, and tip clearance are 76 mm, 0.566 mm, and 1 mm, respectively. Figure 2a presents a 3-dimensional model of the fan.

The whole computational model is shown in Figure 2b. To achieve a reasonable numeric accuracy, it is divided into its different parts. The computational domain is composed of the axial flow fan, fan housing, air sampler inlet pipe, outlet pipe for aerosols, and aerobiology chamber, as shown in Figure 2b.

Grid generation

The physical model of the aerobiology chamber comprises several components with very different geometries. Because of the complicated geometry, unstructured tetrahedral grids were adopted for the whole computational domain. Grids of different sizes were generated for different components and then connected to form the whole geometry. The computational meshes of the aerobiology chamber were divided into 2 zones: rotating zone and stationary zone. Special attention was paid to the geometry and meshing of the fan, with the greatest emphasis on the blades and root of the blades. The rotating zone was a cylindric mesh with 531,218 cells, as shown in Figure 3. Meshes of the surfaces of the axial flow fan



(a) Meshing of axial flow fan surface (b) Rotating cylinder

(c) Cut view of the volume meshing in rotating cylinder

Fig 3. Grid generation in rotating volume.

are also shown in Figure 3. The stationary zone contained 1,125,612 cells.

Several versions of the computational mesh were generated to test the grid independence. Results of this study of the grid are shown in Figure 4. The volume flow rates for cases 3-6 were almost the same. Because case 3 had the lowest computational costs, it was considered the optimum grid number. For a mesh with 1.04 million cells, the maximum cell skewness was 162, and with mesh size of 1.65 million cells, the maximum cell skewness decreased to 154. Thus, the mesh density had an effect on the results for the control simulation case.

Solver

Steady and unsteady simulations

Considering the rotating speed of the axial fan, the airflow was assumed incompressible.[27] The 3-dimensional incompressible Navier-Stokes equations and the k–ε Realizable model were used to model the effects of turbulence on the flow field. The enhanced wall function was used for boundary layer calculation. The second-order upwind differencing format for the convection terms of each

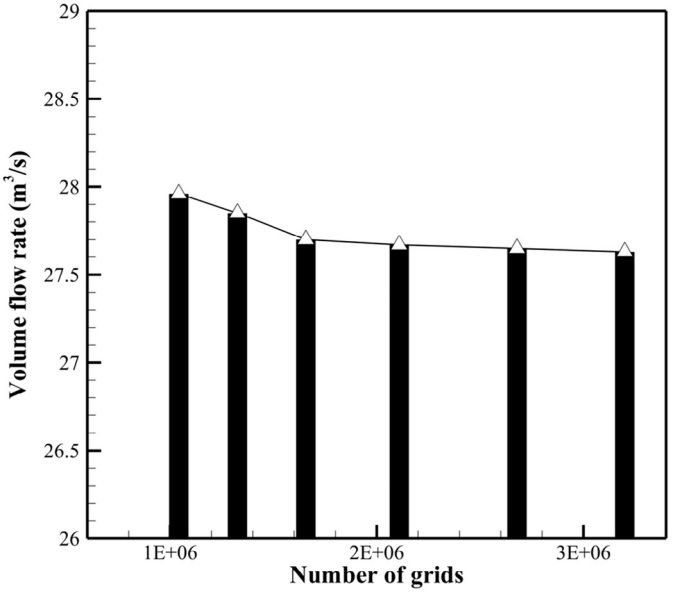


Fig 4. Mesh independency of aerobiology chamber with fan working at 2,500 rpm.

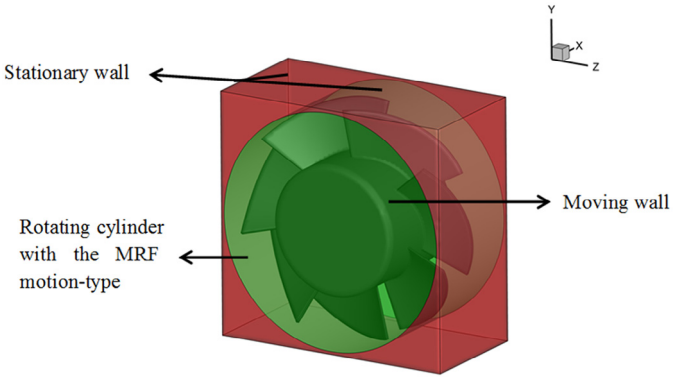


Fig 5. Boundary conditions of fan and its housing. *MRF*, multiple rotating reference frames.

governing equation was adopted, and the second-order accuracy was maintained for the viscous terms. The pressure-velocity coupling was handled by the SIMPLE algorithm for steady solutions and SIMPLEC for unsteady solutions. Because of the large number of computational cells and the possible presence of dynamic effects due to fan rotation, the convergence was satisfied with the criterion of 1×10^{-5} and, in some cases, with the criterion of 5×10^{-6}.

Boundary conditions

The inlet and outlet faces of the fan were set to the interior. No-slip condition was applied on the solid walls. In this simulation, it was assumed that the walls had zero velocity relative to the adjacent fluid. The flowing domain was divided into 2 parts: rotating body and flowing channel. A rotating reference frame was applied to the rotating region around the propeller fan. Different angular velocities were assigned to the rotary zone in the multiple rotating reference frames. A fixed reference frame was applied to the static regions. The conformal interfaces were used for rotor–stator interfaces to accelerate computation speed and improve accuracy. Figure 5 illustrates the boundary conditions of the fan and its housing. Also, the walls of the aerobiology chamber were regarded as stationary.

Turbulence models

Reynolds number was defined based on the fan radius and rotational speed as:[27]

$$\mathrm{Re} = \frac{R^2\omega}{\nu} \tag{14}$$

Where R, ω, and ν are fan radius, rotational speed, and kinematic viscosity, respectively. The Reynolds number of the airflow at a rotational speed of 2,500 rpm was 60,136, which represented a turbulent flow. That is, the existence of the fan as a rotating machine caused a turbulent flow in the chamber. In such a flow, the terms representing turbulence stress should be modeled and added to Navier-Stokes equations. A turbulence model of k–ε Realizable was used to analyze the flow disturbance in the aerobiology chamber. When the fan is operating, its induced momentum is crucial to the airflow and turbulence predictions. Therefore, a low Reynolds number variation of the k–ε Realizable model was used. Flow was solved in 3 rotational fan speeds to select the best velocity for producing uniform flow. The turbulence effects on the particles were accounted for using the discrete random walk model.[27] In addition, it was assumed in the simulations that the particles would rebound to the air after collision with any solid surface.

Postprocessing of the simulation results

The particle trajectories were tracked at different times after particle injection. The nature of the Eulerian-Lagrangian simulation provided for tracking every particle parcel in the flow field at any time.[4] Each parcel that contained a large number of particles was mathematically symbolized as a point in the Eulerian-Lagrangian simulation and represented as a dot in the postprocessed results.[4]

Five different planes passing through the center of the chamber were considered in calculating the area-weighted average velocity magnitude (Fig 6). In a state of uniform flow, the average velocity magnitude in different planes should not be significantly different.

To evaluate bacteria distribution inside the chamber, 5 different control volumes were considered. Each volume was a cube with the dimensions 1 m × 1 m × 1 m (Fig 7). The mass concentrations of particles and the number concentration of particles were calculated. In this study, the number of parcels within the control volume was counted manually. Then, based on the number of parcels, the particle concentrations (number and mass) were determined.

Simulation cases

Twelve configurations of fan position, angle, and velocity were considered (Table 1). The fluid flow was studied in each case with and without injection of aerosolized bacteria. The bacteria distribution and airflow were compared to find the case that could best produce uniformity. To study the effect of furniture on the airflow and bacteria distribution, basic bedroom furniture (ie, a bed, a chair, and a desk) was added to the chamber. The bacteria distribution was then compared with that in an empty room.

RESULTS AND DISCUSSION

Figure 8 compares the 3-dimensional pathlines of the aerobiology chamber for cases 3 and 12. Figures 8a, 8c, and 8d show a vortex, which is not desirable for uniform airflow, whereas Figure 8b is the only case showing no vortex. Such a comparison made between the 3-dimensional pathlines of all cases defined in Table 1 found state 1 (cases 1, 2, and 3) to be the only state with no vortices. This implied that there was uniform airflow when the fan was sitting at a 45° angle in the middle of 1 side of the chamber.

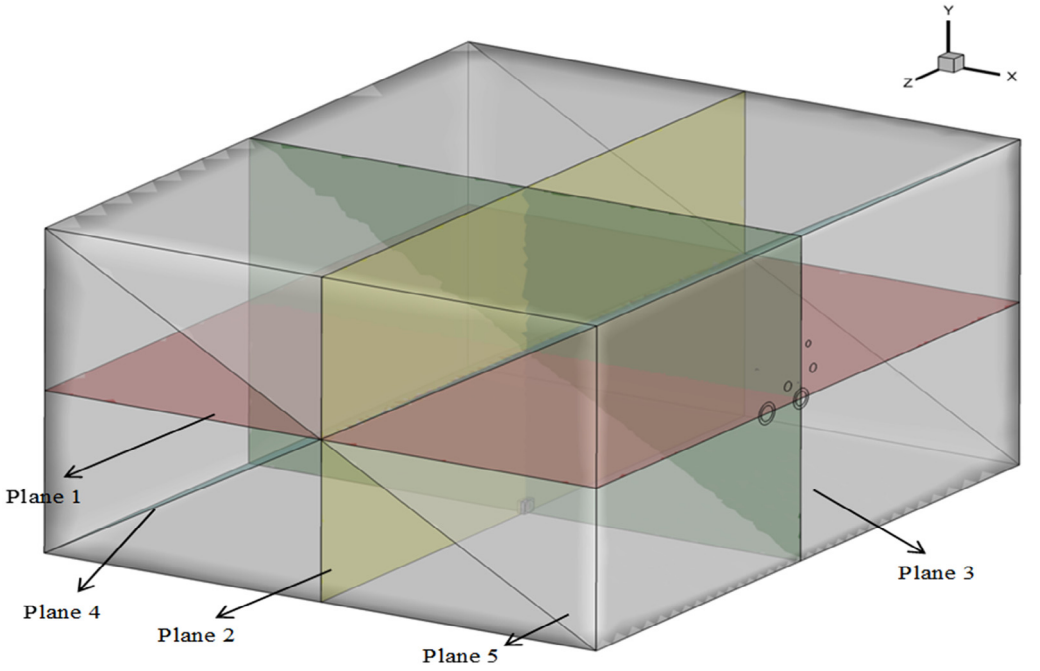


Fig 6. Locations of 5 different planes passing through the center of the aerobiology chamber.

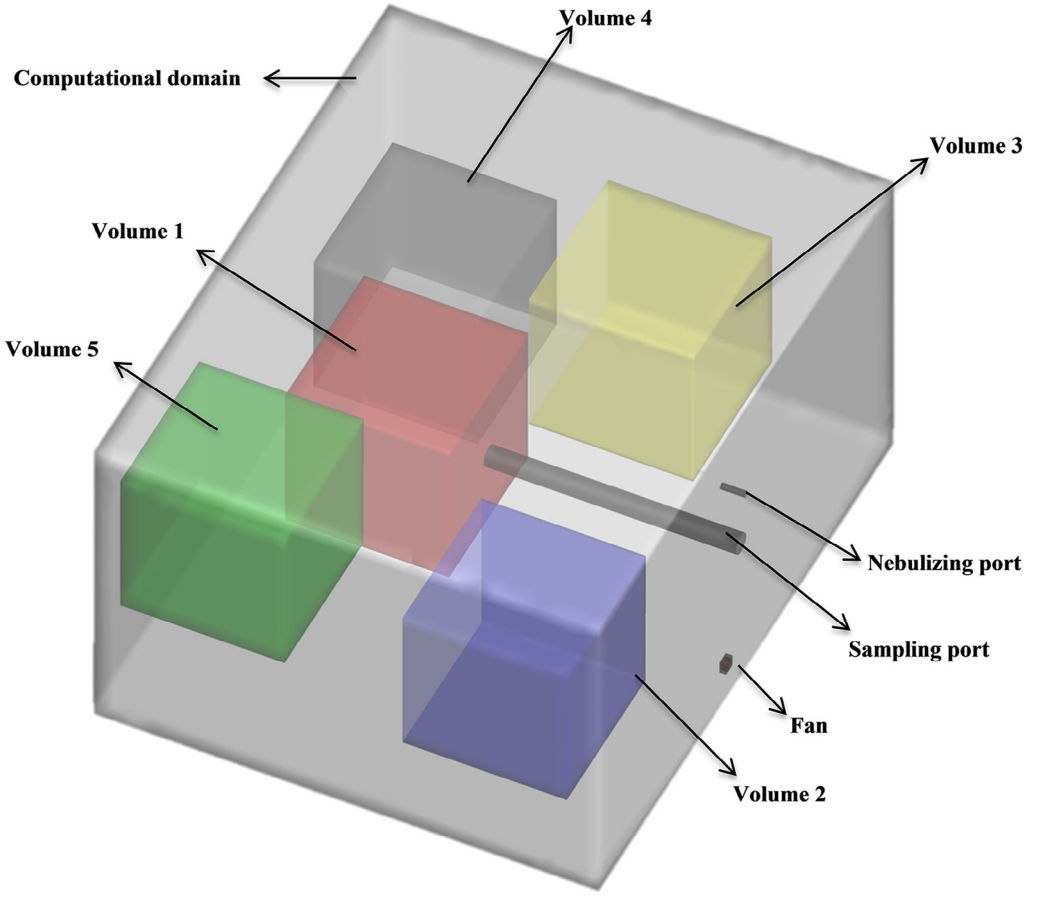
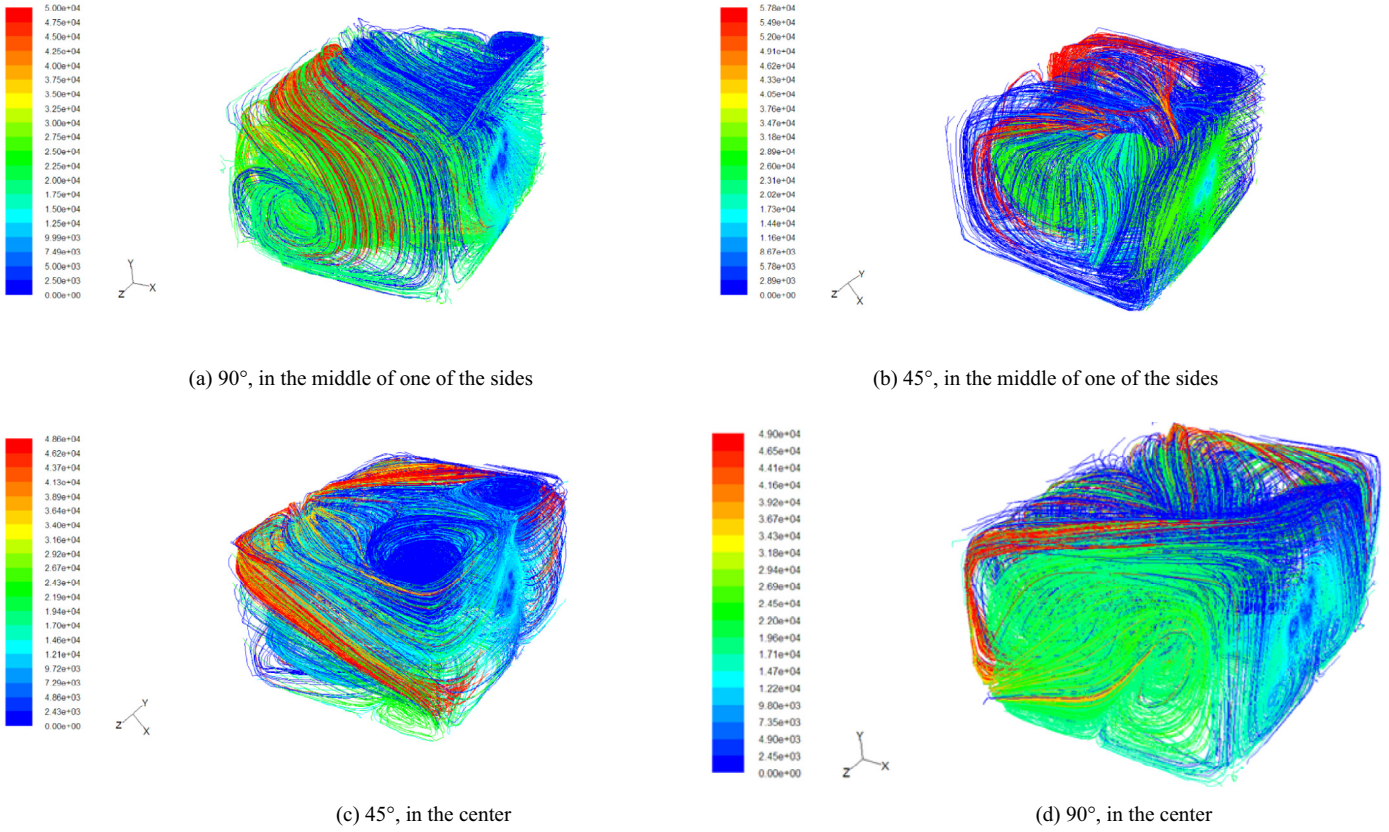


Fig 7. Computational domain and 5 volumes that were considered as samples.



(a) 90°, in the middle of one of the sides

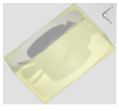
(b) 45°, in the middle of one of the sides

(c) 45°, in the center

(d) 90°, in the center

Fig 8. The pathlines of the aerobiology chamber for the velocity of 2,800 rpm.

Table 1
Different combinations (cases) of position, angle, and speed of the muffin fan

State	Case	Rotational speed (rpm)	Angle	Position
1	1 2 3	2,300 2,500 2,800	45° 	In the middle of 1 of the sides
2	4 5 6	2,300 2,500 2,800	45° 	In the center of the chamber
3	7 8 9	2,300 2,500 2,800	90° 	In the center of the chamber
4	10 11 12	2,300 2,500 2,800	90° 	In the middle of 1 of the sides

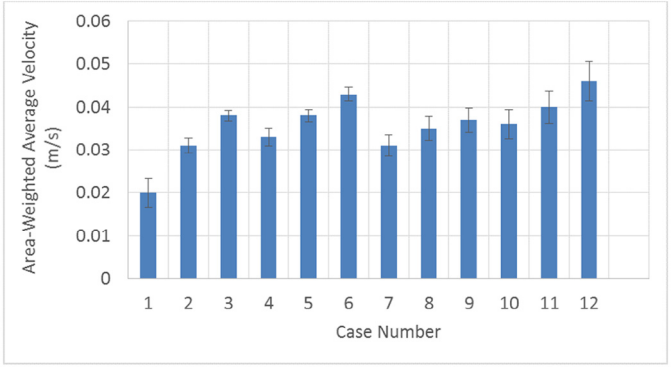


Fig 9. Average and standard variation of area-weighted average velocities on 5 different planes for 12 cases.

To have a better quantitative comparison between states, area-weighted average velocities were calculated on 5 different planes (Fig 9). The average and coefficient of variation (CV) of area-weighted velocities on 5 planes were calculated for each case and are reported in Table 2. Case 3 of state 1 had the smallest CV (6.5%), implying that the fan created the most uniform airflow when positioned in the middle of 1 side of the chamber at an angle of 45° and a speed of 2,800 rpm.

Bacteria were nebulized into the chamber through a port for 600 seconds at a rate of 5,000 CFU/min. For each of the 12 cases, the average of particle concentration in 5 volumes and its CV were calculated 600 seconds after completing the nebulization process (Table 3). Figure 10 shows the average particle concentration in the 5 volumes analyzed and the corresponding standard deviation. Case 3 had the lowest CV, implying that the bacterial distribution in this case was the most uniform. This is in line with our finding from analysis of the area-weighted average velocities. The small standard deviation and CV between the 5 volumes implies that, after 900 seconds, bacteria would be distributed uniformly inside the chamber,

Table 2
Area-weighted average velocity magnitude on 5 different planes for different cases

	State 1			State 2			State 3			State 4		
Case	1	2	3	4	5	6	7	8	9	10	11	12
Speed (rpm)	2,300	2,500	2,800	2,300	2,500	2,800	2,300	2,500	2,800	2,300	2,500	2,800
Plane 1	0.015	0.026	0.036	0.032	0.038	0.043	0.029	0.032	0.035	0.031	0.034	0.039
Plane 2	0.017	0.029	0.036	0.033	0.039	0.043	0.035	0.039	0.039	0.041	0.045	0.052
Plane 3	0.025	0.034	0.041	0.03	0.037	0.047	0.035	0.038	0.042	0.045	0.050	0.059
Plane 4	0.012	0.030	0.036	0.03	0.034	0.038	0.035	0.039	0.040	0.029	0.032	0.037
Plane 5	0.028	0.034	0.040	0.040	0.042	0.044	0.024	0.026	0.028	0.034	0.037	0.043
Mean	0.020	0.031	0.038	0.033	0.038	0.043	0.031	0.035	0.037	0.036	0.040	0.046
CV (%)	34.02	11.08	6.55	12.49	7.67	7.54	16.06	16.34	14.97	18.84	19.09	20.16

CV, coefficient of variation.

Table 3
Log_{10} colony forming units per meters^3 in 5 different volumes at 900 seconds for 12 cases

State	Case	Speed (rpm)	Volume 1	Volume 2	Volume 3	Volume 4	Volume 5	Average	CV (%)
1	1	2,300	4.685	4.565	4.636	4.562	4.679	4.626	0.64
	2	2,500	4.674	4.568	4.620	4.601	4.688	4.630	0.54
	3	2,800	4.662	4.597	4.630	4.631	4.700	4.644	0.42
2	4	2,300	4.678	4.592	4.661	4.541	4.685	4.631	0.67
	5	2,500	4.663	4.570	4.687	4.555	4.692	4.633	0.71
	6	2,800	4.639	4.566	4.725	4.590	4.709	4.646	0.76
3	7	2,300	4.722	4.551	4.664	4.522	4.676	4.627	0.93
	8	2,500	4.713	4.542	4.706	4.556	4.683	4.640	0.90
	9	2,800	4.692	4.497	4.718	4.575	4.697	4.636	1.03
4	10	2,300	4.747	4.564	4.668	4.544	4.692	4.643	0.93
	11	2,500	4.724	4.536	4.674	4.570	4.6988	4.640	0.89
	12	2,800	4.685	4.528	4.695	4.610	4.715	4.647	0.83

CV, coefficient of variation.

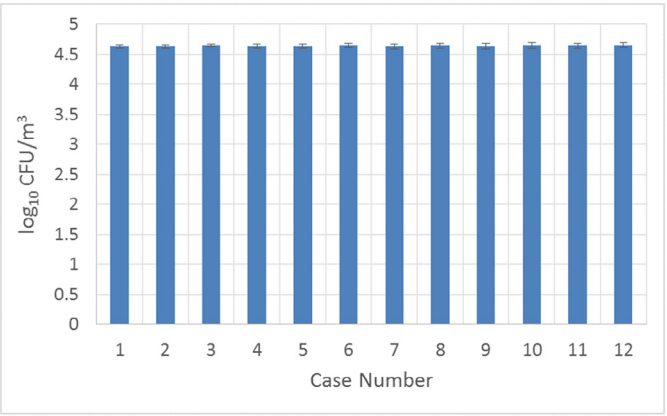


Fig 10. Average and standard variation of bacterial concentration in the 5 volumes analyzed after 900 seconds for 12 cases.

and there may not be any significant difference among the bacteria concentrations in the 5 different volumes analyzed.

The concentration of aerosolized bacteria in each volume was calculated over time for the optimum case (case 3). Table 4 summarizes the results. Figure 11 shows log_{10} colony forming units per meters^3 of samples over the 900 seconds of nebulization of the bacterial suspension into the chamber. The bacteria concentrations in the 5 volumes analyzed were different at the beginning of the process, but the curve of the 5 volumes converged after finishing the nebulization at 600 seconds and reached steady state at 900 seconds. This implies that 300 seconds (5 minutes) of stabilization time after completion of the nebulizing process will result in a uniform distribution of bacteria inside the chamber. That is, the bacteria are uniformly distributed, their concentration has reached a plateau, and the air sampling process can start.

Table 4
Bacteria concentration (CFU/m^3) in 5 volumes

Time (s)	Volume 1	Volume 2	Volume 3	Volume 4	Volume 5
100	1,925	6,865	6,055	2,160	3,195
200	4,315	11,795	10,310	4,965	7,275
300	12,270	21,850	17,405	10,160	16,380
400	21,575	26,960	19,855	17,725	26,120
500	29,475	31,570	33,435	28,430	35,605
600	36,710	40,685	39,695	35,110	41,815
700	48,330	42,740	41,115	38,585	49,575
800	41,860	40,105	43,320	41,010	48,660
900	45,955	39,530	42,655	42,745	50,115

Analysis of variance was performed to determine whether the bacteria concentrations in the 5 volumes analyzed over the time were significantly different. The results showed that the bacteria concentrations were the same at a 99% confidence level ($F_{4,40} = 0.29$; $P = .88$), implying that each of these 5 volumes could be used as a sampling site to calculate the airborne bacteria concentration inside the chamber.

To study the influence of the furniture on bacteria distribution in the chamber, the fan was positioned at the optimum location at a 45° angle at 2,800 rpm (state 1, case 3). Figure 12 shows the schematics of the room with the furniture.

As with the room without furniture, 600 seconds nebulizing time and 300 seconds stabilizing time were considered, and the bacteria concentrations in the 5 volumes were analyzed. The results are summarized in Table 5.

Figure 13 shows log_{10} colony forming units per meters^3 for 900 seconds after initiating bacterial nebulization into the chamber. The concentrations were different at the beginning of the nebulization process but converged during the stabilization time and reached a plateau at the end of the stabilization time.

The bacteria concentrations in the 5 volumes during the nebulization and stabilization processes in the chamber with furniture

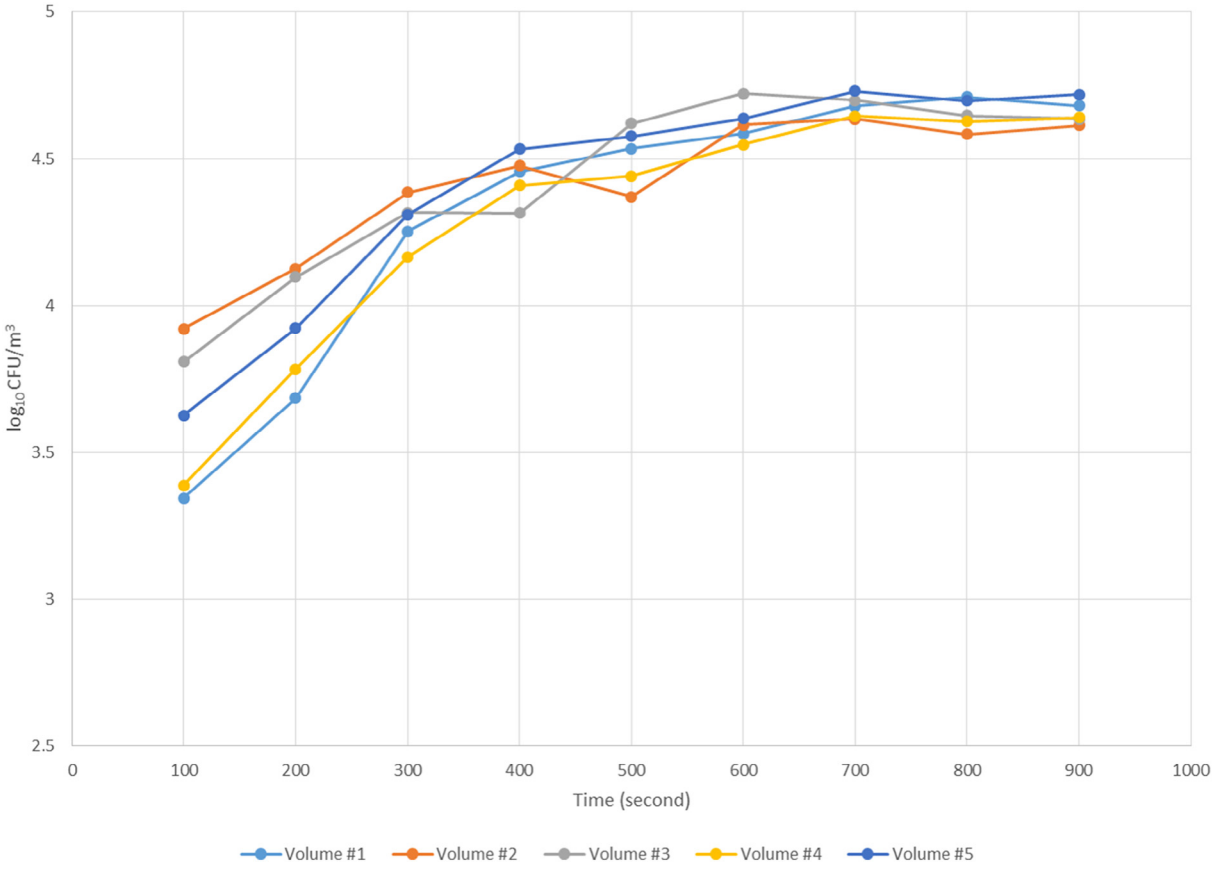


Fig 11. Log_{10} colony forming units per meters^3 in 5 volumes during nebulization and stabilization process. *CFU*, colony-forming units.

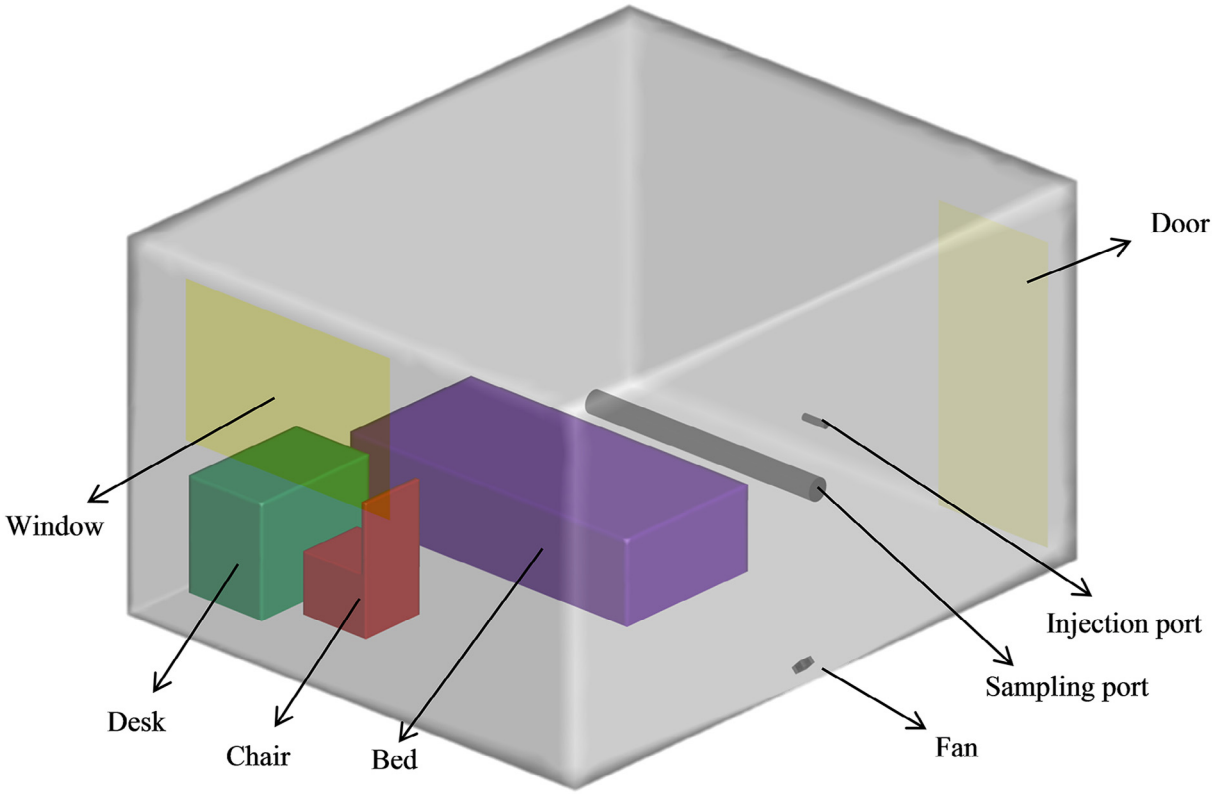


Fig 12. Aerobiology chamber with furniture.

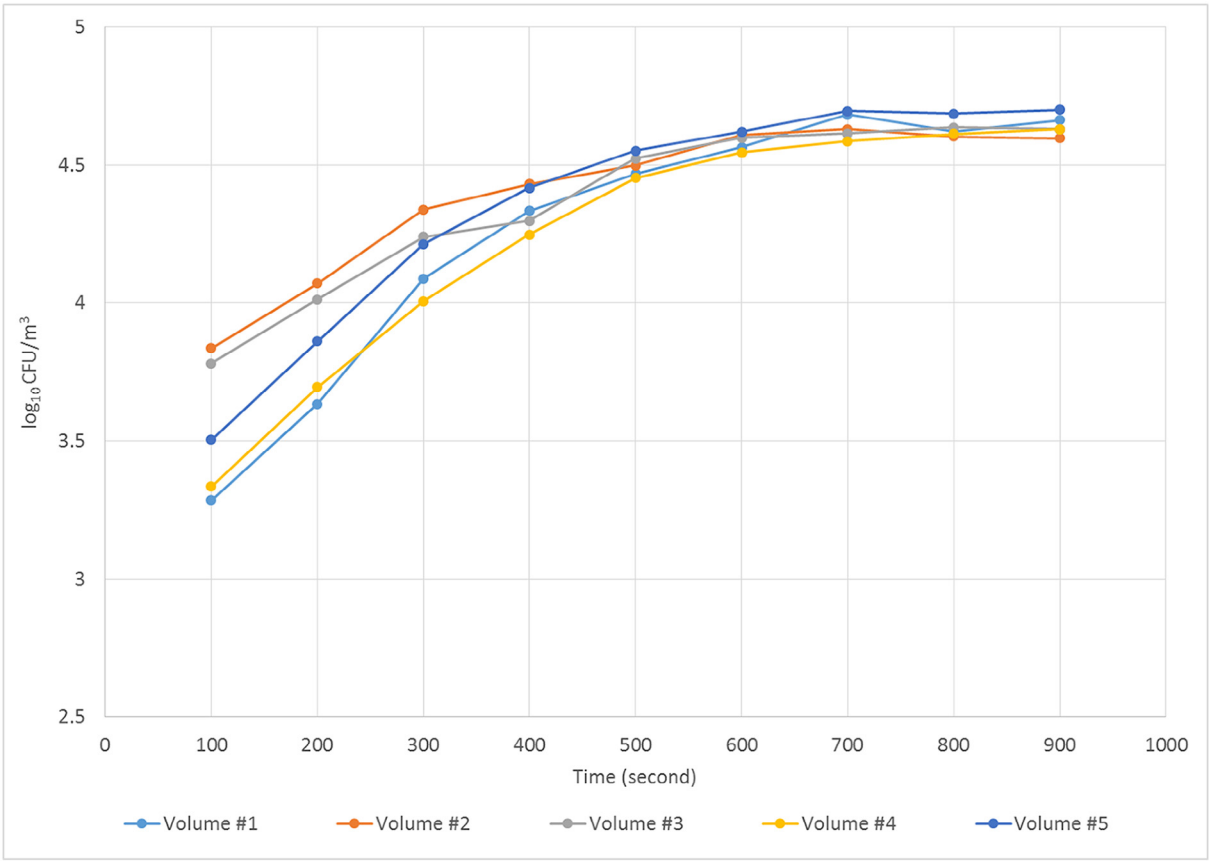


Fig 13. Bacterial concentration in the 5 different volumes analyzed during nebulization and stabilizing times in a chamber with furniture.

Table 5
Bacterial concentration (CFU/m^3) in the 5 different volumes analyzed with room furniture

Time (s)	Volume 1	Volume 2	Volume 3	Volume 4	Volume 5
100	2,215	8,340	6,455	2,445	4,235
200	4,850	13,355	12,530	6,070	8,390
300	17,945	24,250	20,705	14,650	20,445
400	28,550	29,960	20,645	25,685	34,080
500	34,210	23,470	41,645	27,590	37,745
600	38,480	41,280	52,695	35,280	43,415
700	47,730	43,240	49,915	44,230	53,715
800	51,460	38,300	44,310	42,280	49,810
900	47,980	41,005	43,210	43,695	52,430

were compared using analysis of variance. The bacteria concentrations in the 5 volumes were the same at the 99% confidence level ($F_{4,40} = 0.23$; $P = .99$). This implies that, in the presence of the furniture, a single sampling site is sufficient to represent the bacteria distribution inside the chamber.

CONCLUSIONS

Environmental Protection Agency guidelines simply recommend the use of a sealed and empty 800-ft^3 chamber for testing indoor air decontamination technologies, without further specifications on design or operation. However, we considered additional details, such as the time needed for producing a uniform distribution of test bacteria in the chamber with and without basic furniture and the position and number of sites for sampling air from within the chamber. This modeling study, based on CFD, was undertaken to address those issues. Our main conclusions are as follows:

- A muffin fan placed at a 45° angle at the bottom of 1 side of a chamber and operating at 2,800 rpm can provide sufficient air turbulence for uniform bacteria distribution throughout, even in the presence of basic room furniture.
- A 5-minute postnebulization time is sufficient to distribute introduced bacteria aerosols uniformly throughout a chamber.
- Simulating the collection of airborne bacteria from 5 different locations in the chamber indicated that a single site at the center of the chamber was sufficient to provide a representative profile of the concentration of the airborne bacteria.

This information should contribute to further standardization of the design and operation of aerobiology chambers for data generation on the airborne survival of human pathogens, as well as technologies for decontamination of indoor air.

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Major Article

Aerobiology of the built environment: Synergy between *Legionella* and fungi

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Key Words:
Legionella
indoor air
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fungi
biofilms

Background: The modern built environment (BE) design creates unique ecological niches ideal for the survival and mutual interaction of microbial communities. This investigation focused on the synergistic relations between *Legionella* and the fungal species commonly found in BEs and the impact of these synergistic relationships on the survival and transmission of *Legionella*.

Methods: A field study was conducted to identify the types and concentrations of fungi in BEs. The fungal isolates purified from BEs were cocultured with *Legionella* to study their synergistic association. Cocultured *Legionella* cells were aerosolized in an air-tight chamber to evaluate the efficacy of ultraviolet (UV) to inactivate these cells.

Results: *Aspergillus*, *Alternaria*, and *Cladosporium* were the most common fungi detected in samples that tested positive for *Legionella*. After coculturing, *Legionella* cells were detected inside fungal hyphae. The microscopic observations of *Legionella* internalization in fungal hyphae were confirmed by molecular analyses. UV disinfection of the aerosolized *Legionella* cells that were cocultured with fungi indicated that fungal spores and propagules act as a shield against UV radiation. The shield effect of fungal spores on *Legionella* cells was quantified at >2.5 $\log_{10}$.

Conclusions: This study provides the first evidence, to our knowledge, of *Legionella* cell presence inside fungi detected in an indoor environment. This symbiotic relationship with fungi results in longer survival of *Legionella* under ambient conditions and provides protection against UV rays.

BACKGROUND

The evolution in the architectural designs of living spaces and lifestyle changes has resulted in a significant shift of human activities from open-air environments to indoor. Modern living spaces are designed for energy efficiency and controlled air conditions.[1,2] Conditions in the built environments (BEs) used for office or residential purposes are impacted by building design, maintenance operations, and occupant activities. A compromise on these factors can potentially result in sick building syndrome, adversely impacting the health of occupants. The severity of the adverse impact on health appears to be linked to the time spent in such buildings.[3,4]

Indoor air pollution originates from nonbiologic and biologic sources. Bacteria, fungi, pollen, viruses, insect body parts, human squames, animal dander, and bird droppings are the most common sources of biologic contamination in BEs.[5,6] In the indoor environment, fungi are a serious public health threat.[7,8] Fungal species belonging to Ascomycetes, Basidiomycetes, and the anamorphic fungi (Deuteromycota) are the most common causes of allergic reaction in occupants.[8,9]

Legionella pneumophila is the most serious bacterial pathogen associated with indoor environments. Since the discovery of Legionnaire disease in 1976, bacteria of the genus *Legionella* have become a leading public health concern.[10] *Legionella* can be found in air as part of aerosols generated from contaminated sources. It is known to cause Legionnaire disease and Pontiac fever, a milder and self-limiting respiratory infection.[11] Aerosolization is an important component of *Legionella* transmission to the human respiratory system,[12] and it can be accomplished by aerosol-generating systems,

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such as cooling towers, evaporative condensers, plumbing equipment (eg, faucets, showerheads, hot water tanks), humidifiers, respiratory therapy equipment (eg, nebulizers), and whirlpool baths.[12] Continually rising incidences of *Legionella*-associated diseases, and the variety of sources of transmission,[13] highlight the need for understanding the survival and transmission of *Legionella* in BEs.

Factors supporting the survival and growth of fungi and bacteria in BEs have been extensively studied.[14-17] Fungi are able to grow on almost all natural and synthetic materials, especially if they are hygroscopic or wet, and accumulations of dust further facilitate microbial colonization. In addition, increases in temperature and humidity not only facilitate microbial growth but also affect the release of volatile organic compound (VOC).[18-20] The BEs have unique features that separate them from the natural world where microorganisms have evolved over time. Enrichment of particular bacterial and fungal species has been repeatedly shown in BEs under specific conditions.[21-23] The materials, chemical compounds, and physical conditions found in BEs not only provide favorable conditions for selective growth of specific bacterial and fungal species, they also provide niches for unusual interactions among different groups of microorganisms that may not occur in the natural environment.

Although *Legionella* is most prevalent in aquatic environments in BEs (ie, premise water fittings), they have been isolated from nonsaturated media, such as moist soil, dust and potting mixtures.[12,24] In the environment under less than optimal conditions, *Legionella* can survive as parasites of single-celled protozoa (ie, species of *Acanthamoeba* and *Naegleria*).[12] In addition to their ability to live and survive as parasites, they have been shown to develop symbiotic relationships with other microorganisms in aquatic ecosystems[12]; however, no such evidence has been documented for nonaquatic ecosystems. This study focuses on the interaction of *Legionella* with common fungal isolates in BEs and how these interactions impact their mutual survival and dissemination to a susceptible human population.

MATERIALS AND METHODS

Sample collection

During the study period (2014-2015), 52 room-air and 38 vent-air samples were collected from homes and offices in Arizona and California. The room-air samples were collected from the middle of the facility or living room at breathing height, and the vent-air samples were taken from bathrooms and kitchens by positioning an air sampler (PBI SAS-Super ISO Air Sampler; PBI, Milano, Italy) approximately 30.5 cm from the air vent of the surveyed facility or room. Airborne microorganisms settled directly onto Petri plates containing a nutrient medium. The plates were exposed at the sampling points for a specified period of time. The number of microorganisms, expressed as colony forming units (CFU) per cubic meter, was estimated according to the following equation[25]:

$$\mathrm{CFU/m^3} = a \times 10{,}000/p \times t \times 0.2$$

where a is the number of colonies on the Petri plate, p is the surface of the Petri plate (cubic meters), and t is the time of Petri plate exposure (seconds).

For each sampling, the air sampler was actuated to collect the specified air volume (eg, 50 or 100 L of air) from the sampling location. After each sample, the agar plate was removed from the sampler and placed in an incubator at 36°C ± 1°C for 18-72 hours. The bulk sampling of materials, such as settled dust in vents and visible microbial growth on bathroom surfaces, was collected and analyzed as described elsewhere.[26,27]

Identification of isolates

The fungal colonies were identified based on their macroscopic and microscopic characteristics. Macroscopic characteristics included colonial morphology, color, texture, shape, diameter, appearance, and lactophenol staining. Microscopic characteristics included septation in hyphae, presence of specific reproductive structures, shape and structure of conidia-spores, and presence of sterile mycelia.[28] The bacterial cultures were identified based on their colony morphology and growth on differential culture media, and gram-stain reaction.

Legionella *culturing*

All samples were processed, and *Legionella* isolates were cultured according to previously described methods.[29] *Legionella* was cultured on Buffered Charcoal Yeast Extract Agar (Diagnostic Systems, Sparks, MD) medium supplemented with glycine, polymyxin B, vancomycin, and cycloheximide.[30] Culture plates were incubated at 36°C ± 1°C for 72 hours, with an additional 96 hours, if necessary, for full colony formation. Colonies were presumed to be *Legionella* based on morphology.

Microscopy

All dust and biofilm samples were collected carefully without deformation of their surface features. For light microscopy, wet mounts were prepared and observed using an Olympus BX600 microscope (Olympus, Tokyo, Japan). For transmission electron microscopy, samples were embedded in resin and sectioned using a Reichert Ultracut E Microtome. The sections were stained with uranyl acetate and lead citrate and examined using a JEOL 1200EX transmission electron microscope (JEOL, Tokyo, Japan) equipped with a SIA model L3C CCD camera (Scientific Instruments and Applications, Duluth, GA).

DNA extraction and molecular analysis

Legionella samples were analyzed using polymerase chain reaction (PCR) as described elsewhere.[30] DNA was extracted from environmental samples using a ZYMO Research yeast/bacterial DNA extraction kit (Zymo Research, Irvine, CA), and PCR was performed using primers (LpneuF and LpneuR) specific for the *mip* gene. The PCR amplification reaction consisted of 12.5 μL Promega GoTaq Green MasterMix (Promega Biosciences, San Luis Obispo, CA), 10 μL DNA template, and 0.13 μM of each primer to result in a final reaction volume of 25 μL. Gel electrophoresis was performed in a 1% agarose gel containing 0.05 μL/mL of 10,000X Invitrogen SYBR Safe DNA Gel Stain (Life Technologies, Carlsbad, CA) to detect PCR products.

Preparation of spiked samples for aerosolization of Legionella

Pure cultures of *L pneumophila* (ATCC 33153) were obtained from ATCC (Manassas, VA). These cultures were propagated and maintained as recommended by ATCC. The titer of bacteria in each spray replicate was held constant at approximately 10^6 CFU/mL in 0.5× phosphate buffered saline (PBS). Stock concentrations were confirmed by plating a series of 10-fold dilutions of working stock on selective agar and examining for growth after 72 hour of incubation.

Study of synergy between Legionella *and fungi*

All fungal isolates were cultured on plates of Potato Dextrose Agar (BD Difco, Sparks, MD) incubated for no less than 1 week for

sporulation. The spores were harvested using 1× PBS + 0.05% TWEEN-80 (Sigma-Aldrich, St Louis, MO) solution in a shaker (approximately 80 rpm) for 10 minutes. The average spore concentration of the resulting suspension was approximately 10^7 CFU/mL. Culture plates with *Legionella* growth were used to make colony prints on sterile plates of buffered charcoal yeast extract (BCYE) agar without antibiotics. After completion of *Legionella* growth, the plates were stacked, growth surface down, on Potato Dextrose Agar plates with fully grown fungal cultures and incubated (at 27°C, 85% relative humidity) for 2 days. The mixed cultures were harvested using 1× PBS + 0.05% TWEEN-80, as previously described.

Aerosolization of Legionella *cells and ultraviolet exposure*

A spiked sample was sprayed through the port on one side of the aerosolization chamber (air-tight acrylic box with 4 × 3 × 3-ft dimensions) using an air spray gun. The air sampler (PBI SAS-Super ISO; VWR International, Radnor, PA) was placed on the other side to collect 100 L of air from within. To avoid false-positives, the intake of the air sampler was covered during spraying. A BCYE agar plate was placed inside the air sampler to collect bacterial cells from the air. A ultraviolet (UV) C germicidal lamp inside the aerosolization chamber was actuated after each spray. The UV inactivation of aerosolized *Legionella* was tested at various UV doses as a function of different exposure times. Air samples were collected at the end of each exposure, and control samples without UV light exposures were also collected in a similar fashion. The UV light-exposed and control samples were compared to determine the kill caused by UV exposure.[31] After each experiment, the chamber was disinfected using 10% bleach solution.

RESULTS

The concentrations of fungi and bacteria in ambient air in residential and office buildings are presented in Table 1. In homes, the mean concentrations of fungi were 167 and 71 CFU/m^3 during the summer and winter seasons, respectively. Similarly, the highest concentrations of airborne bacteria in homes were detected during the summer. All ambient air samples tested negative for *Legionella* using culture methods. The overall microbial air quality of BEs used for business followed similar trends throughout the study period.

The microbial quality of air coming directly out of vents was tested, and the samples were analyzed for fungi and bacteria using culture methods and for *Legionella* using PCR. The data are presented in Table 2. In residential buildings, the mean concentrations of bacteria in vent air were 635 and 289 CFU/m^3 during the summer and winter months, respectively. Similar variations in the occurrence of fungi in vent air was noted during the study period. Vent dust samples from all sites tested positive for *Legionella* using PCR.

Samples of dust collected from air vent filters and microbial biofilms on bathroom walls were also analyzed for the type of fungi, mesophilic bacteria, and *Legionella*; the results are presented in Table 3. The 4 most prevalent fungi were species of *Aspergillus*, *Alternaria*, *Cladosporium*, and *Penicillium*, with 37.5%, 33.3%, 29.2%, and 25% prevalence, respectively. All samples were positive for mesophilic bacteria, and of those, 14.6% (7/48) tested positive for *Legionella*. The *Legionella*-positive samples were further processed to purify the fungal isolates in them. Two of the purified fungal isolates were identified as *Aspergillus* and *Alternaria*, and a third, an unidentified isolate, was an anamorphic species.

Table 1
Concentrations of bacteria and fungi in the air of office and residential buildings

Setting	Season	Microorganism	Range, CFU/m^3	Mean, CFU/m^3
Office	Summer	Fungi	27-252	152
		Total bacteria	35-202	135
		Legionella	0	
	Winter	Fungi	15-172	98
		Total bacteria	12-153	87
		Legionella	0	
Residential	Summer	Fungi	21-198	167
		Total bacteria	44-298	145
		Legionella	0	
	Winter	Fungi	9-108	71
		Total bacteria	8-92	64
		Legionella	0	

CFU, colony forming units.

Table 2
Microbial concentrations in the air vents of office and residential buildings

Setting	Season	Microorganism	Range, CFU/m^3	Mean, CFU/m^3
Office	Summer	Fungi	145-2,500	552
		Total bacteria	95-3,650	635
		*Legionella**	0-14	3
	Winter	Fungi	35-1,020	329
		Total bacteria	65-1,550	289
		*Legionella**	0-6	0.7
Residential	Summer	Fungi	121-3,740	688
		Total bacteria	65-7,750	701
		*Legionella**	0-17	5
	Winter	Fungi	15-1,250	48
		Total bacteria	83-2,140	121
		*Legionella**	0-6	0.4

CFU, colony forming units.
*Data based on polymerase chain reaction results.

Table 3
Percent prevalence of microbial contaminants in vent dust

Microorganisms	Genera	Prevalence, %
Fungi	*Aspergillus*	37.5
	Alternaria	33.3
	Cladosporium	29.2
	Penicillium	25
	Phoma	8.3
	Stachybotrys	2
Bacteria	Total mesophilic	100
	*Legionella**	14.6

*Data based on polymerase chain reaction results.

Synergistic association between Legionella *and fungi*

The coexisting fungal and *Legionella* isolates purified from dust and biofilm samples were used to conduct in vitro experiments to further confirm their synergistic association. The *Legionella* isolate was cocultured individually with environmental isolates of *Aspergillus*, *Alternaria*, and the unidentified anamorphic species. Observations of mycelial mat samples under the light microscope revealed layering of mycelium surfaces with *Legionella* cells. The *Legionella* cocultured with *Aspergillus* and *Alternaria* remained viable for up to 7 months, whereas coculturing with the unidentified fungal isolate extended its viability to >1 year. Extensive alignment of *Legionella* cells along the fungal mycelium indicated the possibility of symbiotic or parasitic association between these microorganisms. This evidence suggests extracellular biotrophy of *Legionella* on selected fungal isolates. The transmission electron microscope (TEM) analyses of apparently infected fungal mycelia confirmed the internalization of *Legionella* in the hyphae of the unidentified fungal isolate, but not the *Aspergillus* or *Alternaria* isolates. This evidence suggests possibility of endocellular biotrophy of *Legionella* on some fungal isolates.

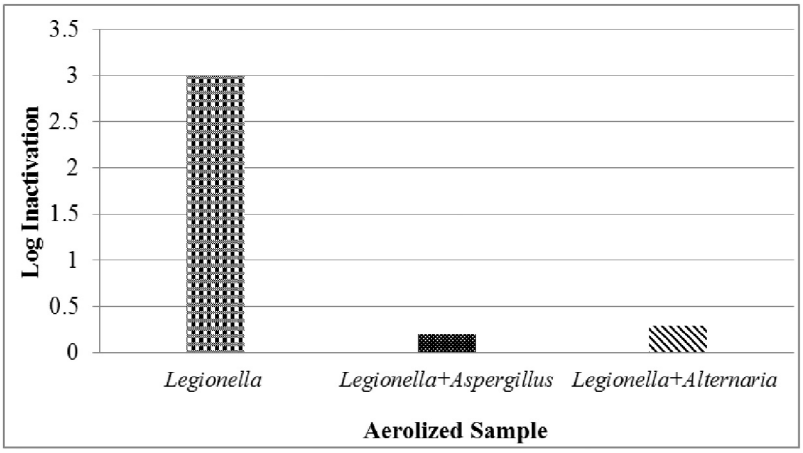

Fig 1. Ultraviolet disinfection of single cells of *Legionella* and coculture with fungi.

Aerosolization and UV disinfection of Legionella *cocultured with fungi*

Three independent experiments were performed using pure cultures of *Legionella* and *Legionella* cells cocultured with the environmental isolates of *Aspergillus* and *Alternaria*. Air samples were collected before and after UV disinfection. The kill effect of UV disinfection on *Legionella* is presented in Figure 1. The $\log_{10}$ reductions of the pure culture of *Legionella* and *Legionella* cocultured with *Aspergillus* and *Alternaria* were 3.0, 0.2, and 0.3, respectively, indicating a protective effect against UV radiation of fungal spores and propagules on *Legionella* cells.

DISCUSSION

This study included sampling sites located in Arizona and California, and comparison of data sets from both states revealed no remarkable differences in terms of microbial concentrations and diversity. BEs are known to contain a complex mixture of bioaerosols constituting fungi, bacteria, and allergens, along with nonbiologic particles.[32] Bioaerosols contribute between 5% and 34% of indoor air pollution.[33] In general, sources of indoor bioaerosols are located outdoors, and intrusion happens through openings in the building envelope (ie, windows, doors). In this study, all sampling sites are located in similar ecogeographic zones (ie, Arizona, Southern California) with similar microbial flora in outdoor and indoor environments.[34] Therefore, a similar microbial complex detected across the study sites is not unexpected. However, variation in BE management and maintenance practices are known to impact the hygiene quality of indoor environments. The housekeeping and maintenance records of participating sites were not available; therefore, a correlation between the microbial quality of indoor environments and stringency of housekeeping and maintenance practices could not be established. In addition, within BEs, the existence of microenvironments in different sections of a building is a common phenomenon. These microenvironment islands are linked with use-based spaces (ie, bathrooms, kitchens), which are known to be characteristically different from the main living spaces.[35] In a previous study, concentrations and types of fungi in bathrooms were found to be different from the rest of a building's sections.[34-36] This study also found higher fungal concentrations in air samples collected from vents in bathrooms and kitchens. In addition, passive sampling (ie, dust and biofilm samples collected by swabbing) resulted in a higher percentage of *Legionella*-positive samples than the active sampling (ie, direct filtration of air). These results are consistent with previous findings from an Italian study in which water was flushed for 2 minutes from a known source of *Legionella* and the surrounding areas were sampled for up to 8 hours using active and passive sampling. Using passive sampling, airborne *Legionella* was detected in twice as many samples as using active sampling.[37]

The environmental stage of *Legionella* is commonly found in soils or in association with detritus and biofilms prevalent in freshwater environments, which are known to have high concentrations of a broad range of heterotrophic bacteria. In oligotrophic environments, the commonly prevalent heterotrophic bacteria can grow more rapidly than *Legionella*. To avoid competition with other bacteria, *Legionella* has evolved into facultative intracellular parasites of free-living protozoa (ie, amoebae). The unusual nutrients required for the growth of *Legionella* are rarely found in the environment[38]; however, as intracellular parasites, they find sequestering niches inside their host, which provide for their special nutritional needs. In indoor environments, aerosolized bacteria eventually land on surfaces. After landing on surfaces, their survival depends on the microenvironment of the sites they land on. For example, bathroom walls and ventilation ducts are known for biofilms containing fungal masses. In this study, *Legionella* was detected in only the samples (biofilms and dust) collected from bathrooms and kitchens. All of the samples tested positive for *Legionella* were also tested positive for at least one type of fungus. Further analyses of these samples confirmed the presence of *Legionella* inside fungal hyphae, indicating the potential of *Legionella* to establish parasitic relationships with saprophytic fungi. During evolution *Legionella* has developed elaborate mechanisms for invading a broad range of host cells and results of this study is consistent with the results of previous studies. Functional Dot/Icm type IV secretion apparatus of *Legionella* allow delivery of effector proteins into their host cells to evade host defenses.[39] Different strains of a single bacterial species may carry variable array of effector proteins, which determine their host range and specificity.[39,40] More than 300 effector proteins have been reported for *Legionella*,[41,42] allowing them to parasitize a broad range of hosts, including human, animals, plant pathogenic fungi, nematodes, protozoa, and amoebae.[43] *Legionella* is known to have enzymes belonging to a novel phospholipase C (PLC) family. Among bacteria, PLC-like proteins are present only in *Legionella* spp and some *Pseudomonas* spp.[39,44] *Legionella* cells rely on Mip, a surface-exposed protein, for the optimal infection of host cells.[45] In addition, Mip is known to influence the secretion of novel PLC-like proteins. Molecular analyses of fungal isolates with *Legionella* internalized in their hyphae revealed a homologous sequence in the conserved motifs of a PLC-like gene found in other *Legionella* hosts (data not presented).

In this study, the most prevalent fungi included *Aspergillus*, *Alternaria*, *Cladosporium*, and *Penicillium*. These fungal genera have been widely documented as common in indoor environments at concentrations similar to the ones recorded in this study.[46-48] On average, healthy adults inhale approximately 0.5 L of air with every breath. Based on average breathing habits, approximately 10 m^3 of air is transported through our lungs every day.[49] Considering the commonly documented microbial concentrations in indoor air, such a large volume of air intake can pose a substantial health risk for physiologically impaired humans. For most healthy individuals, however, the anatomy of the pulmonary system and the clumping-sequestering of microorganisms (ie, increasing particle size) reduce the risk of infection. Nevertheless, reliable comprehensive environmental monitoring data are needed to accurately assess the prevalence of *Legionella* in BEs. This is compounded by the lack of uniform guidance for preventative environmental testing for *Legionella*.[50]

In the environment, microbial clumping not only reduces the risk of fungi intrusion in the lungs, it also imparts a shielding effect against radiation (ie, sunlight, UV, etc). Microbial clumping can create bias in the results of disinfection studies, especially using UV or other radiation. It has been estimated that 61% of the incident radiation of 254-nm wavelength is transmitted through a single microbial cell.[51] A cluster surrounding 2 microbial cells permits only 37% of the incident UV light to reach the third cell. Therefore, clumping of multiple microorganisms as clusters can provide protection against disinfection. Much of the published literature on the microbicidal effect of UV for airborne pathogens is based on studies using one type of mostly monodispersed cells,[31,52] which does not represent a real-world scenario. In this study, *Legionella* cocultured with fungi was aerosolized to test the efficacy of UV. Aggregation of *Legionella* cells with other microbial cells appeared to provide protection against UV, and this information should be considered when planning future studies.

CONCLUSIONS

The importance of evaluating the quality of the air in BEs cannot be overemphasized. The microbial quality in BEs is indirectly influenced by the architectural design and directly influenced by human occupancy, use patterns, and housekeeping practices. The architectural features included in modern buildings for resource efficacy and occupant comfort also serve as a means of creating suitable niches for the enrichment of many types of microbes. From the operational aspect, the microbial quality of indoor air can be an indicator of effective housekeeping and cleanliness and can aid in determining sources of human discomfort. Bathrooms and kitchens were identified as hotspots of microbial communities, from which microbial pathogens can potentially spread to other areas. These areas were found to be appropriate niches for the coexistence of diverse microorganisms, and this is the first study, to our knowledge, to report the synergistic relationship between *Legionella* and fungi in bathrooms. Fungal spores and propagules have high aerosolization capacities. Association with fungal propagules can potentially facilitate *Legionella* cells to become airborne and spread across buildings. In addition, *Legionella* cells clustered with a fungus exhibited high levels of resistance to UV radiation. The results of this study highlight the importance of using appropriate levels of organic material in microbial stocks for the UV disinfection of aerosolized pathogens. The synergistic association noted between *Legionella* and fungi can have significant implications for the successful management of air quality in BEs and emphasizes the significance of environmental testing and monitoring. In the context of the lack of uniform recommendations, various facilities follow the environmental testing program that suits their conditions. The fact that bathroom and kitchens showed the highest level of microbial contamination in residential or commercial use buildings purport to their appropriateness for inclusion in an environmental monitoring and testing program.

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Commentary

The Role of Indoor Air as a Vehicle for Human Pathogens: Summary of Presentations, Knowledge Gaps, and Directions for the Future

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BACKGROUND

This 1-day international workshop on the role of indoor air in the spread of human pathogens comprised 6 invited presentations that were selected to meet a set of objectives. The event was organized under the auspices of and with support from ASTM International (http://www.astm.org/). The City University of New York and the University of Ottawa (Canada) were the 2 academic sponsors, and the financial support for the workshop was provided by RB (Montvale, NJ; http://www.rb.com/) and Microbiotest, a division of Microbac (Sterling, VA; https://www.microbac.com/our-laboratories/sterling-va/). RB also funded publication of the conference proceedings.

Five of the speakers (A. Alum, C. Duchaine, Y. Li, S.A. Sattar, and B. Zargar) are university-associated experts in diverse aspects of research in the aerobiology of pathogens. The sixth speaker (M.K. Ijaz), who represents both industry and academia, also brought extensive experience in the study of airborne viruses and other types of human pathogens (Fig 1).

The first presenter (Dr Sattar) set the stage with a brief historical perspective on aerobiology, with particular focus on the significance of indoor air as a vehicle for human pathogens, and then defined the objectives of the workshop and what to expect from it. The final event of the workshop was a panel discussion that engaged all speakers and the audience, addressing 3 preformulated questions.

Objective 1: Review the role of indoor air in pathogen spread

All presenters addressed this topic from the perspective of their own research. Professor Li gave an overview of how pathogens can spread via indoor air and the potential for airborne pathogens to contaminate environmental surfaces for secondary spread. Dr Alum expounded on the complex microbial ecology of biofilms in the built environment and biofilms in premise plumbing as significant sources of opportunistic pathogens, such as legionellae. Dr Ijaz further elaborated on the complex interactions between airborne pathogens indoors and, in particular, the resuspension of microbial aerosols from contaminated surfaces for further spread by air.

Objective 2: Methods to study pathogen survival in air

Dr Duchaine discussed this topic with specific reference to the use of the rotating drum[1] for the aging of experimentally aerosolized pathogens under different conditions of air temperature and relative humidity and their subsequent inactivation by physical and chemical agents.

Dr Ijaz described a room-sized aerobiology chamber[2] built in conformance with the 2012 guideline from the U.S. Environmental Protection Agency (EPA).[3] He presented original data generated using this chamber that showed the airborne survival of several types of vegetative bacteria that are used as surrogates for human pathogens. He then illustrated and described the use of the chamber to assess commercially available devices claiming decontamination of indoor air. These are the first data of their kind to be generated using an EPA-specified aerobiology chamber.

Objective 3: Means of pathogen recovery from indoor air

This topic was covered only to a limited degree, except for the mention of a biosampler by Dr Duchaine and a slit-to-agar sampler for event-related profiling of viable bacteria and other microbes in air, which was discussed by Dr Sattar, with illustration of its use for direct capture and enumeration of viable airborne bacteriophage MS-2 on a lawn of its *Escherichia coli* host borne on the surface of a recovery medium.

Objective 4: Math modeling to validate and refine test facilities

Dr Zargar used mathematical modeling of the aerobiology chamber, described earlier by Dr Ijaz, to show that (1) the type, location, and speed of the fan was optimal for rapid and uniform

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Conflicts of Interest: None to report.

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Fig 1. Group picture taken at ASTM International's Workshop, "The Role of Indoor Air as a Vehicle for Human Pathogens," held on April 13, 2016, in San Antonio, Texas. Pictured from left to right: Professor Y. Li, Dr A. Alum, Dr B. Zargar, Dr S.A. Sattar, Dr John Mitchell, Dr C. Duchaine, and Dr M.K. Ijaz.

distribution of airborne particulates in the chamber; (2) collection of the air sample from the center of the chamber only was appropriately representative of the distribution of airborne particles throughout; and (3) the simulated presence of basic bedroom furniture in the chamber produced no measureable difference from the distribution of airborne particles measured in the empty chamber. Such modeling can refine the design and use of aerobiology chambers and reinforce the relevance of the test parameters that were defined in the test protocols described by Dr Ijaz and the application of the general guidance given by the U.S. EPA.[3]

Objective 5: Review the ways of indoor air decontamination and report on the current research on this topic

The presentations by Drs Duchaine and Ijaz both addressed this topic by highlighting that (1) robust and field-relevant methods for the testing of indoor air decontamination technologies remain unavailable; (2) human and environmental safety of available and developing technologies are in need of better and verifiable assessment of claims; (3) better test protocols are essential to refining guidelines for premarket assessment of technologies for indoor air decontamination and their eventual registration; and (4) scientifically valid data are needed that demonstrate that proper decontamination of indoor air can prevent or reduce the contamination of environmental surfaces, thereby interrupting the air-surface-air nexus.

Objective 6: Better surrogates for airborne pathogens

Dr Duchaine summarized the suitability of a variety of bacteriophages as surrogates for airborne viruses that are pathogenic to humans, with examples from her group's recent studies.[4] She also reiterated that the use of bacteriophages in studies of aerobiology offers the advantages of human and environmental safety, ease of handling, lower cost, and relatively rapid turnaround of results.

Dr Ijaz's recent data confirmed that *Klebsiella pneumoniae* is less stable in indoor air than *Staphylococcus aureus* and *Acinetobacter baumannii*. The latter is an important emerging airborne pathogen in health care settings and, because of its airborne durability, should be considered for use in aerobiology studies as a gram-negative health care–associated alternative to *K pneumoniae*.

FUTURE DIRECTIONS FOR RESEARCH AND DEVELOPMENT

At present, our understanding of the behavior of airborne human pathogens and the relative importance of indoor air in their spread is in its infancy. This is mainly because of an absence of suitable test facilities and robust and scientifically valid test protocols. Further, efficient and sensitive means for detecting low levels of viable airborne pathogens are absolutely crucial to any field investigations that intend to establish the role of indoor air as a vehicle for a given pathogen.

Recent culture-independent microbial metagenomic studies are helping define the "airborne microbiome"[5] and revealed the ability of viruses to remain airborne indoors viably for extended periods.[6] However, because studies based on metagenomics alone cannot readily distinguish between infectious and noninfectious pathogens,[7] findings must be interpreted with caution with regard to the health impact of pathogens in environmental samples in general.

Further, the health impact of combined or sequential exposure to airborne pathogens and other pollutants, such as chemicals, respirable particulates (eg, $PM_{2.5}$), and allergens,[8,9] must be more clearly understood. Recent studies on the spread of methicillin-resistant *S aureus* in hospitals have highlighted the need for focusing on abiotic and biotic factors through an interdisciplinary approach to mitigating the risk of airborne spread.[10]

Table 1
Continuing challenges in research on aerobiology of human pathogens

Factor(s)	Refinements required
Experimental setup	Space, biosafety, field relevance, ease of control, and monitoring of test parameters
Challenge microbe selection	Representative of airborne pathogens, ease of culture and recovery, stability during aerosolization and in air, and preparation and concentration of culture
Suspension to be nebulized	Safe and standardized soil load representing body fluids, antifoam, and physical tracer (if needed)
Nebulization and particle size distribution	Protection for microbe, generation of aerosols and droplet nuclei, granulometrics, and uniform distribution
Aging and exposure conditions	Control of air temperature and RH, testing at an RH <20%, and harmonization for relevant microbial types
Aerosol collection and sizing	Protection of viability, optimal growth conditions, and neutralization of actives
Assessing decontamination	Proper controls, realistic efficacy criteria for method and device air decontamination technologies, and number of replications
Interpretation of data	Statistical analyses, field relevance, and regulatory requirements

RH, relative humidity.

CONCLUDING REMARKS

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

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

1. Experimental facilities for the study of survival and transport of airborne pathogens (viruses, in particular) remain rare because of a lack of specialized equipment, infrastructure, and technical skills.
2. Practical and standardized processes for recovering viable pathogens from field samples of air remain unavailable, thereby preventing linking of airborne exposure to a variety of infections.
3. Simultaneous or sequential exposures of hosts to airborne pathogens and other harmful substances, possibly combining to create negative health impacts, make risk assessment particularly challenging.
4. Surrogate microbes often used for the study of the aerobiology of human pathogens may be unsuitable for the purpose because of an inability to withstand aerosolization and remain viable in air. A major research need is the identification of appropriate bacteriophages because they are attractive surrogates for the study of airborne human pathogenic viruses. This definitely is a topic for further investigation.
5. Despite the increasing number and variety of technologies claiming indoor air decontamination, robust and scientifically valid protocols remain unavailable for their validation.

Meaningful approaches to resolving the knowledge gaps that are described here will require the joint efforts of microbiologists, architects, and specialists in indoor air handling systems.

Table 1 summarizes the research needed to refine the study of aerobiology of human pathogens.

Other areas of evolving significance are microalgae[11] and enteric protozoa[12] as airborne pathogens. Although it is relatively easy to detect, by molecular means, the presence of a wide variety of microorganisms in indoor air, relating such data to actual health risk remains a major challenge. As always, answers to these and other questions will require better and longer-term research funding.

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Commentary

Workshop on "The Role of Indoor Air as a Vehicle for Human Pathogens": A Panel Discussion

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The primary objective of this panel discussion was to seek input on the following 3 questions from the speakers at the workshop. Comments and questions were also entertained from the members of the audience.

PANEL DISCUSSION

Question 1: Considering the potential advantages of microbial decontamination of indoor air, what criteria should be used to select a given technology?

Dr C. Duchaine: Technologies of interest will depend on the situation and the point source of microbial contamination, for example, a person coughing in an emergency room, a patient vomiting in a hospital ward, or a student vomiting in a classroom. The intervention of choice must be relevant for the site, for instance, a hospital versus a shopping mall. Portable indoor air decontamination devices may be used in smaller areas, such as classrooms, but devices that would allow global decontamination (eg, at a hospital or a shopping mall) would be more challenging. When possible, routine and continuous air treatment may be better than an on-site intervention.

Dr Y. Li: Technologies that are effective for decontamination of circulating air in different situations would be more desirable. One example is the use of UVGI in air circulation ducts if the potentially generated ozone can be effectively removed to avoid its entry into occupied zones. The technology of choice must be safe when people are present, while also being energy efficient. Further, it must not only be effective, but also scalable to suit the site of use. The results of testing in an aerobiology chamber can be much different than those in a large area, such as a hospital or a large shopping mall, where there are on-going changes in the air quality parameters. The debate continues as to whether indoor air for breathing should be treated in the same fashion as water for drinking.

Dr S.A. Sattar: Yes, it is certainly true that waters for drinking and swimming are routinely decontaminated, but not indoor air in most settings! Titanium dioxide filters in ventilation systems could be very valuable as they can work on an ongoing basis without adding any chemicals to the air. However, in situations of very close contact between 2 people, it is virtually impossible to prevent exposure to airborne pathogens. There is an invention though that claims personal protection against airborne pathogens and other contaminants based on 2 thimble-sized cups with filters to be placed inside the nostrils [http://www.breathecleanerair.org/]. I believe that this device requires independent testing and perhaps additional refinements.

Audience member 1: Much of what is being discussed has been done before using algorithms and flow rates at the UNLV [University of Nevada Las Vegas] with the U.S. EPA [Environmental Protection Agency] data. A peer review team consisted of NASA [National Aeronautics and Space Administration] and the EPA radon/asbestos particulate team. There is a rich body of data from the IEST [Institute of Environmental Sciences and Technology, Arlington, IL] on clean room standards and aerospace standards that you should be reviewing. For the space industry, particle exposure testing was performed using microbeads as surrogates. Airflow could be studied without the issue of contamination/decontamination of the chamber. The mass and shape of material contaminants can affect results and the decisions made. Did you consider this? Solutions can be found in other disciplines, and many materials do not require monitoring.

Dr Sattar: It is unfortunate that this original work is not more readily accessible. The 2012 EPA guidelines do not reflect this background information, which could have impacted chamber design.

Audience member 2: Decontamination is too strong of a term unless complete kill is demonstrated. Microbial reduction is a better term.

Dr Sattar: For indoor air quality, you can differentiate between physical removals by filtration versus killing. Decontamination captures both physical removal and killing of microorganisms. Reduction also could be used to describe the situation.

Audience member 2: In real life, it is very difficult to decontaminate a room completely, as microorganisms will be entering continuously through doors, windows, and cracks. Unless the entire building is sealed, there always will be low levels of organisms present.

Dr Sattar: The purpose of air decontamination is not to sterilize the room. The emphasis is on decontaminating the air and not the surfaces. By decreasing the microbial content in the air, you would hope to reduce the contamination present on surfaces as well.

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Although the EPA guideline mentions it, one should avoid the term "sanitization of air" and use "decontamination" instead. "Sanitization" is a nebulous term and is difficult to define. That term is not used in Europe.

Audience member 3: As with any technology, there can be unforeseen consequences, for example, as observed with the high-efficiency plumbing. What are the potential consequences of decontamination of indoor air?

Dr Li: Some microbes are good and some are bad, and the goal should be to remove those organisms that are contaminants in the air. People should not be encouraged to decontaminate ordinary spaces unless known contaminants exist. Air decontamination is important in health care settings, such as for hospital spaces. Overdoing microbial decontamination may have secondary environmental impacts. During the SARS [severe acute respiratory syndrome] outbreak in Hong Kong, significant environmental decontamination and community hygiene measures were associated with a reduction in the reported incidence rates of other respiratory infections during that time.[1] On the other hand, overuse of disinfectants can lead to more pollution in the wastewater.

Dr M.K. Ijaz: No matter what the technology, it has to be safe. In case there are safety concerns to humans, the space being decontaminated should not be occupied during treatment. With regards to opening of doors and re-introduction of microbial contaminants, the data I presented addressed this by showing that a proper indoor air decontamination device can continually deal with ongoing fluctuations in indoor air contamination. No one is suggesting to eliminate all microbes from the air, and, from a practical point of view, it is impossible. The focus is on risk reduction, and a suitable air decontamination device can lower the risk of airborne spread of pathogens.

Dr B. Zargar: One needs to consider the location of the indoor air decontamination device in a given room and also make sure that the existing air-handling system is working properly. Occupants' exposure to potentially harmful bacteria should thus be reduced as much as possible. CFD [computational fluid dynamics] is used widely to simulate the behavior of airborne microorganisms in various settings. To the best of our knowledge, this is the first time that CFD has been used to assess the design and performance of an aerobiology chamber to test the behavior of microorganisms in indoor air. Since the size of airborne particles is an important consideration, we have chosen for our simulation particles in the range of 0.5 to 5 μm in diameter.

Dr Sattar: It is a valid concern not to introduce technologies that will have unforeseen consequences. Good communication is required among engineers, designers, architects, and microbiologists. We are becoming wiser in introducing technologies that are safe and sustainable. Air decontamination goes beyond removing and killing bacteria. Is it conceivable that air decontamination also might remove allergens and pollen in addition to pathogens? Technologies that have a broad base, beyond just microorganisms, might be a more attractive proposition to the consumer.

Audience member 2: If we continually operate the device, we do not know the impact of reduced exposure to pathogens on our immune system. What is the effect of living like this?

Dr Ijaz: Microbial load will be reduced, but contaminants are continually reintroduced. Contamination is minimized, but you do not remove it completely. This is a question of risk reduction.

Audience member 4: What was the size of the chamber you used for testing and modeling of data?

Dr Sattar: Our chamber was built according to the EPA guideline, but was slightly >800 ft^3, or about 24 m^3.

Dr Zargar: The minimum chamber dimensions suggested by the U.S. EPA guidelines are 10 feet × 10 feet × 8 feet (3.048 × 3.048 × 2.44 m).

Audience member 5: One needs to consider the size of the test chamber versus the size of the device. For example, UV [ultraviolet] decontamination is related to energy level, distance, and time. Is it fair to test a device designed to decontaminate a large area and a device meant for a small area in the same sized chamber? Does this give an advantage to the device designed for larger rooms? There are practical concerns with this.

Dr Ijaz: The test chamber built at the University of Ottawa is in accordance with the EPA guideline and is designed as an average-sized room. The chamber is useful for screening technologies. For larger areas, field studies would be required to prove the effectiveness of the technology.

Dr Sattar: Look at HVAC [heating, ventilation, and air conditioning] systems for understanding. The equipment is scaled to the size of the building. It is a matter of engineering rather than the device, and one has to cater to the needs of the home. It is possible that more than one device will be required for decontamination.

Audience member 5: Scale the device to the chamber?

Dr Sattar: Yes.

Dr Ijaz: The research team at the University of Ottawa has already tested a number of different devices. The key question is "how fast and how frequently can the chamber air be processed through the device?" One of the devices produced a 3-log_{10} reduction in experimentally aerosolized bacterial challenge in 45 minutes, while another one required >3 hours to achieve the same level of bacterial reduction. Therefore, the performance of the 2 devices was drastically different.

Audience member 2: The chamber was designed specifically for microbial decontamination of a room, and the EPA provided the size as a standard. You need to know the size of the room you want to decontaminate. Future products would need to be labeled for the size of the room and tested in this size.

Audience member 1: There are many companies active in this area. In the agricultural field, there are ISO [International Organization for Standardization]- and WHO [World Health Organization]-approved protocols for decontaminating facilities such as chicken and egg houses. Construction and other materials in the room may absorb or adsorb the chemicals used to decontaminate the air, and this must be considered. ASHRAE [American Society for Heating, Refrigeration, and Air-Conditioning Engineers] has many standards in this area. There are several guidance documents and standards available in other areas upon which one can draw.

Dr Zargar: The range of airflow can be adjusted for different room sizes, for example, a higher speed for larger rooms and a lower speed for smaller rooms. Instead of building different-sized chambers for testing different devices, mathematical modeling can be used to map the result of experiments in rooms of different sizes.

Audience member 6: Which came first—clean air or fresh air? Is there a standard definition?

Dr Sattar: The EPA standard is a 3 log_{10} reduction in the level of viable bacteria. One needs to know the baseline values to determine if a technology can result in a 3-log_{10} reduction in a specified period of time.

Question 2: What should be the essential elements for an experimental aerobiology facility in terms of the biosafety level and the size and configuration of the test chamber?

Dr J. Mitchell (moderator): This question has been partially answered in the discussion so far.

Dr Sattar: We sought input from the CDC [U.S. Centers for Disease Control and Prevention] as well as from the NIH [U.S. National Institutes of Health]. Based on the feedback, if we aerosolize a biosafety level-2 (BSL-2) organism, then the testing must be conducted in a BSL-3 laboratory. Although we have been using a BSL-3 facility thus

far, our university has recently decided to downgrade that facility to BSL-2 to reduce maintenance and operating costs and to cut down on the paper work for periodic certification as a BSL-3 lab. The decision was based on risk assessment considering that proper staff training and use of personal protective equipment can minimize exposure to the microbes being aerosolized. However, there is still no general consensus on the biosafety containment level needed to work with aerosolized BSL-2 organisms.

Dr Duchaine: If we aerosolize a BSL-2 organism, we use a double-containment strategy so exposure is limited.

Dr Li: It is important to ensure that the chamber is fully mixed so that the organisms are uniformly dispersed and the environmental conditions remain constant. Organism survival is a function of temperature and humidity. Within a chamber that is not uniform, the organism can be dispersed into different environmental conditions. Different-sized chambers are employed for different purposes (eg, VOC [volatile organic compound] release). This is probably reflected in the fact that a typical room or building size does not exist, as different indoor environments are of different sizes.

Question 3: The U.S. EPA guideline (2012) for testing indoor air decontamination technologies recommends the use of specific strains of Staphylococcus aureus *and* Klebsiella pneumoniae *as surrogates for airborne gram-positive and gram-negative vegetative bacterial pathogens, respectively. Are they the most suitable for the purpose? Also, should additional surrogates be considered for other classes of airborne pathogens, such as viruses, fungi, mycobacteria, and spore-forming bacteria? If yes, what desirable attributes should we consider in selecting such surrogates?*

Dr Sattar: This is a crucial aspect where we want to consolidate our views on selection of surrogates. This is not only important for aerobiology but for environmental microbiology as well.

Dr Duchaine: Several types of surrogates will be required, as they all do not behave in the same manner. Eventually, one will need to work with the real contaminating organism to confirm the response. It is important to validate that the surrogate behaves like the actual contaminating organism.

Audience member 3: Dr Alum, in your *Legionella* studies, what was the inoculum level used and does it represent the typical situation that occurs during an outbreak?

Dr Alum: The level of *Legionella* used in the studies was 10 cfu [colony forming units]/m^3. For nonoutbreak–associated cooling towers, we have found, with limited studies, that the *Legionella* numbers were very low, <10 cfu/mL. In cooling tower water associated with outbreaks, the *Legionella* concentration is relatively high.

Audience member 3: In selecting surrogates, do we also need to consider expected numbers present in the targeted situation?

Dr Sattar: Yes, but we always build into the study a certain redundancy and a certain higher level of performance. Hence, the level observed in the actual field situation may not be the level used in testing. The product label has to have assurances built in, so this is the reason we use a higher level of test organisms. It is absolutely right that we need to have surrogates that closely represent those present in air. Consider surrogates in 4 categories: vegetative bacteria, spore-formers, fungi, and viruses. Microalgae are a new area that I cannot comment on yet. If this is a health concern, it should be considered as a fifth category.

Audience member 1: Algae were studied in the 1970s and shown to be an allergen, producing an immune response. Since microalgae exist in the normal environment, I would suggest we keep it on the list as a fifth category.

Audience member 5: There are a lot of data for surface disinfection that suggest a general hierarchy of resistance. Do you see the same hierarchy for aerobiological studies? When norovirus is detected in air from a contaminated area, what is the source? It is less likely from direct breathing, but rather from vomit or fecal aerosolization of norovirus. If norovirus is primarily transmitted by touch, then is it important to decontaminate the air if air transmission does not cause infection?

Dr Duchaine: The source is the patients themselves. The sampling device was present where the actively emitting patients were, and they may have vomited or had diarrhea. There may be up to 10^{12} virus particles per gram of diarrheic feces, and this would be part of the aerosol. Surfaces are cleaned, but if an airborne route exists, this explains how other patients some distance away become infected. One way to prevent spreading of infections is to decontaminate the air. For the first part of your question, droplet nuclei need to come in contact with the product for an appropriate period of time. Unlike surface testing, the air decontaminant contact time is longer.

Audience member 5: How do you compare the resistance levels of the different categories of organisms (bacteria, virus, and fungi) in air?

Dr Sattar: You need to develop comparative data, but one would expect differences in comparing liquid disinfectants to air treatments. For liquid disinfectants, there is a larger volume to infectious agent ratio. In air, the ratio is not the same, and the testing dynamics are different. Therefore, one cannot compare liquid disinfection testing to air decontamination. There is a need to consider surrogate organisms for each of the 5 categories because there are practical and ethical issues to deal with in working with actual pathogens. For example, *M* [*Mycobacterium*] *tuberculosis* can spread by air, but it is a relatively slow-growing pathogen and difficult to obtain in viability titers high enough for aerosolization. Also, how can we grow enough of the SARS virus to contaminate an aerobiology chamber? If regulators allow the use of surrogate organisms for testing environmental surface disinfectants, why can their use not be extended to air testing?

Audience member 7: We should not limit ourselves to key pathogens, such as respiratory, as less known ones can become a problem. Could infection or airflow be modeled through adjoining rooms or halls, rather than in a chamber? That might be beneficial to better align with the real world.

Dr Zargar: Modeling of a furnished chamber can be used as the starting point for simulation of a hospital room. Modeling of the ventilation system in any such setting would be crucial.

Audience member 2: Following up on the hierarchy question, AD [Antimicrobial Division of the U.S. EPA] for registration purposes allowed 60% glycol products to have an air sanitization claim. Data are required to support this, so registrants must provide the data. This testing is in its infancy. As more data become available, a hierarchy for air decontamination can be developed. Selecting surrogates also is still in its infancy and currently is supported by historical selection.

Mr Rubino: It is time to close the session, and I would like to thank ASTM International for supporting this workshop. Special thanks to Dr Sattar, Dr Mitchell, Dr Ijaz, and the rest of the team, as well as to the speakers.

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