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6.08 Indoor Microbiome and Airborne Pathogens

Marcus HY Leung, Xinzhao Tong, and Patrick KH Lee, School of Energy and Environment, City University of Hong Kong, Hong Kong, China

Glossary

Amplicon high-throughput sequencing targeted DNA (or RNA) sequence analysis of a specific known gene region (or intergenic region) for the identification of microorganisms within the microbiome. Some of the most common amplicon targets are the bacterial 16S rRNA gene and the fungal intergenic spacer one region (ITS1). Amplicon sequencing uses primers that specifically target the flanking regions to be sequenced.

Built environment a non-natural environment created and designed by humans for maximal comfort during human activities. Built environment (BE) usually refers to indoor environment but can also be outdoor (e.g., parking spaces and urban parks that are not enclosed).

Commensalism symbiotic relationship between microorganisms in which one microorganism benefits from the symbiosis, whereas the other does not benefit nor has detrimental effects.

Culture-dependent methods methods in microbiome research that are based on the cultivation of microorganisms from an environmental sample. Culture-dependent methods rely on the viability of the microorganisms and the laboratory growth conditions.

Culture-independent methods methods in microbiome research that are based on direct analysis of nucleic acids extracted from any environmental sample without the need of microbial cultivation. Culture-dependent methods can survey both viable and non-viable populations within the environment.

Microbiome/microbiota microbiome represents the entirety of genetic material present in the microbiota, which is the entire collection of microorganisms within an ecosystem.

Shotgun metagenomics high-throughput sequencing sequence analysis of all genetic materials (DNA and/or RNA) in a particular environment to assess the structural attributes and functional potentials of the targeted microbial ecosystem.

6.08.1 Introduction: The Need to Understand the Indoor Microbiome

The rapid growth of the global human population has prompted worldwide urbanization and the need for immense infrastructural improvements. Indoor environments (or built environments, BEs) have become a predominant habitat for modern individuals, as urban individuals spend the majority of their times in BEs, mostly in homes, workplaces, schools, public transports, hospitals, and other indoor spaces. Concomitant with this rapid growth and expansion of BEs, there is also a quest to maintain and protect the well-being of indoor occupants. Individuals in BEs co-exist with a myriad of bacteria, fungi, viruses, and microbial parasites such as mites. These microorganisms together constitute the microbial community (or the microbiome) of the BE.

Early interests into the BE microbiome were focused on specific pathogens and how they were spread within indoor environments. However, recent efforts have begun to unravel the indoor microbiome as a whole, examining the different effects of indoor environments on the community with broader and greater ecological focuses. Recent advances in culture-independent high-throughput sequencing have further catalyzed the bloom in indoor microbiome investigations, allowing the detection of hundreds and thousands of microbial species in a single indoor air or surface sample. As a result of high-throughput sequencing, the research community now appreciates that the breadth of microbial diversity within BEs are several orders of magnitude higher than previously reported using culture-dependent methods.[1] In addition, while earlier phylogenetic marker gene-based targeted sequencing studies have identified the microbial community members within BEs, studies using shotgun metagenomics sequencing have further surveyed the functional potentials of a microbial community, thus enabling one to gain new insights into metabolic, virulence, and resistance potentials of the air microbiome.[2,3]More recent efforts have also linked microbial communities and chemical compositions detected in various indoor environments, in attempts to detect relationships between microbiome and potential microbial metabolites and other chemicals present in BEs.[3]

Given the rapid development in BE microbiome research, an overview regarding the past and current state of research is timely and warranted. Therefore, in this chapter, the importance of understanding the indoor microbiome from an ecological, environmental, and clinical perspective will be discussed. This will be followed by a brief historical account of BE microbiology, describing the earliest works looking at microorganisms in indoor settings. A description of some of the common contemporary methods applied to study the indoor microbiome, from sampling to data analysis, will also be provided. Different compartments of the indoor microbiome, such as air-, surface-, and dust-associated microbial communities, as well as building designs and other factors that may be important in driving differences in the BE microbiome will be summarized. While most of the current BE microbiome works using high-throughput sequencing pertain to bacteria and fungi, studies involving other members of microbial life will also be briefly discussed. The subsequent description of the transmission of microbiomes within the BE, including the risk of pathogens and their spread within BEs, will underscore the importance of understanding the BE microbiome. Finally, future outlooks in BE microbiome research will be presented.

6.08.2 Importance of Understanding the BE Microbiome

Scientists for decades long to elucidate the relationships between human beings and their surrounding physical elements, which can also be related to the biotic attributes of the natural environment, as well as climate and other chemical and physical factors. Similarly, microbial ecology, defined as the study of microorganisms in the environment, has been fundamental in understanding natural biochemical and geochemical processes on earth, providing insights into how humans can use the knowledge of the microbial world in different ecosystems for the improvement of our health and well-being at the individual and societal levels. New scientific works under the theme of microbial ecology, some of which also adopting the more recent term "microbiome" and/or "microbiota," are published on a daily basis, testaments to both the importance of understanding the repertoire of microbial communities around us and the prevalence of such research being conducted globally.

The appreciation for research focusing on the BE microbiome has been evident from articles outlining pressing research questions and methodological considerations when conducting BE microbiome works.[1,4,5] Indeed, the man-made nature of BEs make them unique environments compared to any other ecosystems, with a very diverse range of buildings in terms of design, location, occupancy, ventilation or maintenance. These building properties can shape distinct microbiomes in similar BEs. The core BE microbiome in general consisted of microorganisms with outdoor environmental origins, as well as contributions coming from indoor occupants such as humans and pets. Moreover, activities of occupants may also play major roles in shaping the BE microbiome.[4,6,7] In addition, depending on the operational function and purpose of the BE (from single-family residential buildings to commercial buildings), building characteristics and indoor environmental factors including temperature, humidity, and airflow rates (as controlled, for example by a heating, ventilation, and air conditioning (HVAC) system) are adjusted to maximize occupant comfort. These factors are associated with changes in indoor microbial communities, but their roles may be modest compared to that of BE design, occupancy, and outdoor influences, depending on the BE (see **Section 6.08.6**).

Given the large amount of time we spend indoors, understanding the BE microbiome will also provide insights into the risk of pathogen transmission between occupants, and how transmission is mediated by contacts between different individuals, as well as between individuals and indoor surfaces. Multiple studies have demonstrated that cohabitation affects the composition and structure of skin-associated microbial communities, and that residential cohabitants share more similar skin microbiomes suggesting that the BEs at least have an indirect effect on an individual's microbiome.[6] The rapid spread of severe acute respiratory syndrome

(SARS) virus within a residential complex in Hong Kong in 2003 (see Section 6.08.8) is a clear example for the need to understand the roles of different BEs, environmental and occupancy factors in transmitting pathogens, so that such transmissions can be prevented in the future.

6.08.3 Historical Context

Prior to the use of culture-independent methods and sequencing of nucleic acids present in the environment using new-generation sequencing platforms, majority of the indoor microbiome works conducted in the last century and the beginning of this century involved culturing microorganisms (mostly bacteria and fungi) in laboratory growth media. Microbiologists typically were able to cultivate common occupant-associated bacteria including species of *Staphylococcus* as well as environmental members including *Bacillus*.[8] However, culture-dependent methods inherently present numerous limitations in BE microbiome research. Firstly, only a small fraction of naturally occurring microorganisms can be cultivated in laboratory growth conditions. Hence, the microbial diversity obtained from culture-dependent methods has been greatly underrepresented. Indeed, studies that include both culture-dependent and culture-independent works unanimously highlight the extended microbial diversity detected via culture-independent methods, including the identification of potentially pathogenic species, which were otherwise undetected using culture-dependent methods.[8,9] Lee et al.[8] also uncovered a much wider range of bacterial diversity in a daycare facility using 16S rRNA gene clone libraries, and most of these bacterial species were not detected when the same samples were cultivated. Notably, through 16S clone sequencing, the authors have established that pseudomonads were considered to be a core group of microorganisms in the childcare facility, even when members of this group were only sporadically detected on agar plates.

The inability for most indoor microorganisms (cultivability potentially as low as 0.01%[1]) to grow under laboratory growth conditions inevitably presents a biased and potentially distorted view of the number and relative proportions of microbial species co-existing in BEs. For example, cultivable organisms such as *Staphylococcus* are likely to be overrepresented in indoor settings, in light of our current knowledge of the extended diversity of the indoor microbiome. More importantly, the results obtained from culture-dependent study likely depend on the cultivation conditions employed (e.g., recipe of growth medium, incubation temperature, etc.).[8] Therefore, inter-laboratory comparison of culture-dependent results is challenging and not meaningful if the culture growth conditions between studies are different.

The earliest culture-independent sequencing works on the BE microbiome involved the construction of clone libraries, followed by Sanger sequencing after genomic DNA (gDNA) extraction from environmental samples. While Sanger sequencing has granted building scientists with an expanded view of the breadth of microbial diversity present in BEs, Sanger sequencing has been superseded by the pyrosequencing method, developed by 454 Life Sciences (Roche, Branford, USA), due to the reduced labor time, cost, and increased sequencing depth. With the advent of pyrosequencing, an increased number of BE microbiome studies had been conducted, and these previous works gave scientists early insights into the effects of building design, occupants and their activities, geography, climate, and time on indoor bacterial and fungal communities.[1,4] While pyrosequencing was the preferred sequencing method of choice for a period of time since its introduction, pyrosequencing has lost its competitiveness to Illumina sequencing (because of its increased sequencing depth and hence reduced cost per nucleotide), which is currently the most commonly used sequencing platform for microbiome analysis. Illumina sequencing is the current sequencing platform of choice for analyzing the BE microbiome, especially when shotgun metagenomics sequencing is employed.[2–4] More recently, third-generation high-throughput sequencing platforms, such as the Pacific Biosciences and the Oxford Nanopore technologies, allow for longer read length (comparable to those offered by Sanger sequencing, making full-length 16S rRNA gene sequencing possible, see Section 6.08.4.3), thereby potentially enabling more accurate and specific identification and community analyses of BE microorganisms.

Despite the increased breadth of diversity that can be detected by high-throughput sequencing, culturing may still provide valuable information regarding the cultivable members of the indoor microbiome. For example, while metagenomics sequencing has the ability to detect and identify the genetic basis of potential drug resistance,[2,3] culture work can undeniably demonstrate the resistant phenotype. Using agar plates containing different antimicrobial compounds, Afshinnekoo et al.[2] reported that drug-resistant bacteria were prevalent in various surfaces of the New York subway system. Therefore, culture-independent methods will complement rather than entirely replace culture-dependent methods, as the inclusion of both culture-dependent and culture-independent methods will undoubtedly present a more comprehensive characterization of the BE microbiome.

6.08.4 Laboratory and Bioinformatic Methods to Characterize the BE Microbiome

The steps required to undergo sequence analysis necessary for microbiome research include sampling, nucleic acid extraction, amplification (for marker gene studies), as well as sequencing and bioinformatic analysis. In order to effectively address a research question pertaining to the BE microbiome, careful considerations in each of these steps are crucial. As described previously[4] and summarized below, variations in any of the steps will result in differences in the observed microbiome data. Currently, there is little standardization in the methods adopted by different BE microbiome studies, making inter-study comparison and metaanalyses difficult. As in any scientific work, when one begins to design a microbiome research experiment, it is important to thoroughly consider the possible methodological options, and select one that is the most appropriate for addressing the intended research question. The best practices for the different steps associated with conducting microbiome research works have also been reviewed recently.[5]

6.08.4.1 Sampling

The initial step in BE microbiome analysis is the collection of samples, which can come from the air, surface and water at any location associated with the BE. Depending on the source, different sampling methods are required. For example, air samples can be collected actively using different bioaerosol samplers that draw air directly from the immediate environment.[4] Alternatively, the BE air microbiome can be inferred by collecting settled dust samples or dusts and particulates from HVAC filters.[4] The selection of air sample type has been shown to be associated with differences in the detected microbiome.[4] Similarly, the type of surface swab and the swabbing protocol also influence the recovery of microorganisms from BE surfaces.[10] These results highlight the challenges in comparing studies adopting different sampling methods, as the majority of BE microbiome studies involve the use of only one sampling method.[4] More importantly, careful consideration of the appropriate sampling protocol for each study should be exercised.

6.08.4.2 Nucleic Acid Extraction

The extraction and purification of genomic DNA from the collected sample is one of the most crucial laboratory steps in microbiome analysis, and maximizing yield is especially important with low biomass samples such as those collected from BEs. A number of commercial kits are available for routine extraction of different sample types (e.g., air filter, surface swab, water, etc.) collected in BEs. As extraction kits are not necessarily DNA-free,[4] the inclusion of controls in the extraction step is important for the effective detection and removal of contaminating sequence data at later steps.

Metabolically active cells of the BE microbiome can be detected using the propidium monoazide (PMA) dye, which can penetrate cells with compromised membranes and bind to its DNA, thereby inhibiting PCR amplification.[11] Alternatively, sequencing of complementary DNA (cDNA) as a proxy for RNA to detect the metabolically active members of the BE microbiome, as well as RNA viruses, may be facilitated by the use of RNA extraction kits. However, these kits are typically designed to remove genomic DNA present in the total genetic pool. Therefore, in order to simultaneously survey both DNA and RNA populations of the microbiome, collection of duplicate samples (one for DNA and one for RNA extraction) or applying a co-extraction protocol is required.[12] Other detection methods, such as bromodeoxyuridine staining and stable-isotope probing, may also be employed in the future to identify active components of the indoor microbiome.

6.08.4.3 PCR

The majority of BE microbiome studies involving amplicon sequencing to date target the bacterial 16S rRNA and/or the fungal ITS marker genes. For bacterial 16S rRNA gene sequencing, due to the short read length of the Illumina platform, sequencing is often limited to a fragment of the 16S rRNA gene. Specifically, one or two of the nine hypervariable 16S rRNA gene regions are sequenced. The V1-2 and V4 regions are commonly targeted for microbiome works of the BE.[4] Despite their popularity, primers targeting these regions do not encompass the entire ribosomal gene, and full length 16S rRNA gene sequencing may be preferred. Specifically, third-generation long read length sequencing platforms such as Pacific Biosciences and Oxford NanoPore sequencing technologies may allow the standardization of full-length 16S rRNA gene sequencing, thereby bypassing observed community variations that arise from sequencing partial 16S rRNA gene regions. Additionally, full-length 16S rRNA gene sequencing will also increase the resolution of the detected sequences to species and strain levels within the BE microbiome.

6.08.4.4 Bioinformatic Analysis

A suite of bioinformatic tools are available for microbiome analyses, and they have been adopted and used for BE microbiome characterization. Some earlier BE microbiome works involved the use of mothur,[13] an open-access software package first introduced in 2009. Mothur enables sequence read quality control and processing, taxonomic classification, as well as community composition analyses and additional tasks and functions. Despite the early introduction of this tool, mothur is still currently used in studies investigating the BE microbiome (references of such studies can be found in Ref. 4) and is continuously updated with additional features.

Quantitative Insights Into Microbial Ecology (QIIME) is perhaps the most widely used analytic package in BE microbiome research.[14] Similar to mothur, QIIME contains a suite of wrapper tools that enables sequence quality processing, taxonomic classification, and various community diversity and compositional analyses. One associated tool, SourceTracker,[4] provides a Bayesian approach in estimating the proportions of the microbiome coming from particular sources. Since its introduction, QIIME and SourceTracker have been used widely in BE microbiome studies, providing insights into the relationships between the microbiomes of the BE and that of occupants such as the importance of occupant skin and gut microbiota in feeding the indoor microbiome (depending on building characteristics). In addition, SourceTracker results also point to the outdoor microbiome being an important source of the BE microbiome.[4] Recently, the originally developed QIIME has been updated to QIIME2 that features a more streamlined user interface and expanded analytical capabilities for more accurate microbiome analyses. As with mothur, QIIME2 is continuously updated with new features.

6.08.4.5 Laboratory and Bioinformatic Pipelines for Shotgun Metagenomics Analyses

Metagenomic shotgun sequencing enables the characterization of the metabolic, virulence, and resistance potentials of the entire microbial community without the need of PCR amplification. Also, compared to targeted sequencing of phylogenetic or functional

marker genes, a greater amount of nucleic acids is required for shotgun sequencing. Bioinformatic tools for the analyses of shotgun metagenomic data can be broadly divided into read-based (where taxonomic and functional profiling of short sequence reads obtained from the BE are performed) or assembly-based (where taxonomic and functional characterizations are performed after short reads are assembled into contigs or metagenome-assembled genomes). Shotgun sequencing in general, as well as bioinformatic tools available for metagenomics data analysis, have been reviewed recently.[15]

6.08.5 Different Components of the Indoor Microbiome

Microorganisms in BEs are dependent on dispersal from outdoor environments and indoor occupants (including humans, pets, and plants), coupled with the selection pressure imposed by architecture design and building function, as well as occupants' behaviors and activities. In this section, we review microbial communities in three key indoor reservoirs (air, surface and dust) and highlight the roles of outdoor air and human occupants in shaping the indoor microbiome. Given the predominance of bacteria and fungi in BEs, they are discussed in more details than other members of microbial life.

6.08.5.1 Indoor Air Microbiome

Airborne microorganisms in indoor environments comprise a diverse community originating from soils, plants, humans, animals, and water sources. In addition to a few ubiquitous species, most environmental microorganisms encountered indoor are confined to a specific location of the building. In other words, it is less likely for two BEs in close proximity to harbor identical airborne microorganisms of environmental origins. This is primarily due to differences in microbial community of outdoor air, which has been predicted by source-tracking analysis as a major source of indoor airborne microorganisms across a wide range of BEs such as dwellings, classrooms, offices, and public transit.[4,16]Having said that, there may exist a "core indoor microbiome" across building types, including taxa belonging to genera commonly associated with occupants and those that may be pathogenic.[17]

The dispersal of outdoor microorganisms may be regulated by the mode of ventilation applied in BEs. In a previous hospital study, chloroplast DNA was found to be significantly less abundant in the air of mechanically ventilated rooms than those that were naturally ventilated, which was thought to be removed by the filters of mechanical systems.[18]

In addition to the migration of outdoor air, some indoor pathways and reservoirs may affect the load of airborne microorganisms in BEs. Human occupants emit microorganisms predominantly from the skin, and occupants have long been recognized as a major source of indoor airborne microorganisms.[6] The emitted microorganisms not only increase the microbial load, but also affect the composition of airborne microbial community. Common inhabitants of various parts of the human body can be detected in BEs.[4] Moreover, the indoor space was imprinted with a personalized microbial cloud which could be used to predict the occupancy of specific human hosts.[1] However, when the indoor space was well ventilated and sparsely occupied, the indoor airborne microbial community closely mirrored outdoor air, with modest microbial signature from human body.[4,16] These results suggest that the contribution of outdoor air and human occupants to indoor air microbiome is associated with the occupancy density and frequency and ventilation strategy.

In many BEs, an important source of airborne microorganisms is the growth of fungi or molds. When moisture is available in BEs, fungal spores can germinate and form colonies where thousands of new spores are produced. In damp houses, visible mold growth is often associated with increased concentration of *Cladosporium* spores, and exposure to *Penicillium* and *Aspergillus* spores may trigger asthma in children.[19] Moreover, the relative proportion of moisture-related airborne fungal species tends to increase in dwellings with limited ventilation, low air exchange rate and high humidity.[19] Given the adverse health effects of molds and spores, a review paper contends that fungi can be used as an indicator of indoor air quality,[19] but there is no consensus yet on the level of indoor airborne fungi that signifies contamination. To minimize the growth of molds, ventilation is an effective strategy in damp BEs. In addition, developing strategies such as environmental-friendly mold removal products to eliminate harmful molds or spores from BEs will benefit human health.

6.08.5.2 Indoor Surface Microbiome

BE surfaces provide an ideal interface for particle deposition. Indeed, deposition is an important mechanism, but not necessarily the final fate of airborne microorganisms. It is known that airflow can deliver particles onto surfaces, while such air movement can also cause particles already settled to be resuspended in the air. In a previous indoor study, Thatcher et al.[20] examined the correlation between airflow speed and particle deposition rate. The results suggest that the deposition rate of all particle sizes (0.5–10 μm) increased with increasing air speed (5–19 cms^{-1}), with larger particles showing greater effects than smaller ones. In addition, at a given airflow speed, the deposition loss–rate coefficient (this term is defined as the number of particles depositing on the total surface available for deposition per unit time, and is expressed in units of s^{-1}) increased with particles size (i.e., larger particles are more likely to deposit compared to smaller particles). Despite the lack of direct evidence on microbial transmission between air and surface within BEs, it is plausible that most microorganisms on BE surfaces, especially those of environmental origin, are deposited from indoor air, or are the result of direct intrusion from outdoors.

The type of surfaces may also select for specific microorganisms. Within an indoor space, distinct microbial communities can be found on different types of surfaces, which are influenced by occupants' behaviors and activities. Skin-surface contact is a major route for microbial transmission, with transfer events reported between hands and routinely touched surfaces such as doorknob as well as bare-foot and floor.[6] As a result, indoor surfaces may be imprinted with personalized microbial fingerprints that are traceable to a specific body site. Likewise, microorganisms can also be introduced indoors by shoes coming into contact with floors. For example, linoleum floors in rural residences were found to be contaminated with fecal indicator *Escherichia coli* originated from outdoors with boots acting as viable transporters.

In addition to direct transmission of microorganisms between human and BE surfaces through physical contact, microbial transmission is also possible through indirect mechanisms. For example, floors in many BEs generally harbor abundant skin-associated bacteria, which is thought to be the result of skin shedding and microbial deposition following aerosolization.[4] Moreover, surface microbial community can be affected by occupants' deliberate actions such as cleaning practices. The load of bacteria and viruses on contaminated surfaces was significantly reduced through a single wipe with a wet fabric, and the treatment with disinfectant resulted in additional reduction of all pathogens probably by the means of inactivation. However, a recent study by Kwan and colleagues[21] demonstrated that skin-associated microorganisms can return and re-establish onto surfaces in schools within days following routine cleaning, and suggested that schools may require more frequent cleaning to reduce the transmission of potential pathogens between occupants.

6.08.5.3 Indoor Dust Microbiome

Indoor dust is thought to be the time-integrated assemblages of particles which have previously been airborne and consists of organic and inorganic materials with microorganisms in relatively high abundance. Most microorganisms are unlikely to grow in dust unless adequate moisture is available. In most BEs, microorganisms in dust are considered to be mainly dispersed from outdoor air, as the surfaces where dust deposits are unlikely to be cleaned frequently and/or are in little or no direct contact with human occupants. Indeed, indoor dust harbors abundant microorganisms of soil and plant origin, with microbial community composition varying upon changes in the local outdoor microbial reservoir. Hence, it is expected that the indoor dust microbial community is largely shaped by outdoor environmental conditions when a large geographic scale is considered. Consistent with this expectation, a global survey of household dust reported that phylogenetically distinct fungal communities were identified for each region, and the fungal communities in temperate zones were more diverse than those in the tropics.[22] Within a smaller geographic scale, the seasonality pattern may be reflected in the dynamics of indoor dust microbial community when a long time scale is considered.

In addition to environmental taxa, human-associated microorganisms may also deposit on indoor surfaces after being released as aerosols via skin shedding or respiration. Barberán et al.[23] reported that household dust contained abundant bacteria with human dermal, vaginal and fecal origins, and the number and sex of occupants greatly affected the bacterial community in household dust. In contrast, the composition of indoor dust fungal communities was predictable across different geographic and climatic regions, with minimal influences from human occupants.[23] Occupant activities such as walking can cause the resuspension of settled dust, which is considered an important mechanism for aerosolizing indoor microorganisms.[16] Overall, indoor dust harbors a highly diverse microbial community where bacterial members are strongly affected by human occupants, while fungal members are largely mediated by geography and climate.

6.08.6 Different Factors in Shaping the BE Microbiome

The BE microbiome can be shaped by myriads of building, anthropogenic, environmental and physical factors. Here we summarize some of the major factors governing indoor microbial community compositions, which include ventilation, spatial property, occupancy, vegetation, geography, and climate. Some of these factors have also been reviewed previously.[6,7]

6.08.6.1 Ventilation Mode

Ventilation is a key strategy in architectural design, regulating the entry of ambient air into indoor space. Natural ventilation generally provides sufficient air exchange rate and outdoor airborne particles can easily penetrate openings and leaks of the building envelopes to enter indoors. Mechanical ventilation is commonly equipped with in-system or in-duct filters so that outdoor particles that are larger in sizes are restricted from entering the indoor space. Thus, the influence of outdoor microorganisms on indoor microbiomes is expected to vary according to the ventilation strategy adopted in the BEs. Consistent with this, airborne bacterial communities in naturally ventilated rooms resembled adjacent outdoor air, while distinct microbial community was found in rooms with mechanical ventilation. In addition, mechanically ventilated rooms harbored less diverse microbial communities than naturally ventilated rooms.[6] Similar to indoor air, the type of ventilation was also shown to affect the structure of indoor dust microbiome. However, it is worth noting that an improperly maintained ventilation system can serve as an amplification and dissemination site for microbial pathogens.[16] Overall, ventilation plays a crucial role in shaping the diversity and composition of indoor microbiome, and regular maintenance (i.e., cleaning practices) is required to improve the air quality of BEs.

6.08.6.2 Spatial Characteristics

Within a BE space, the spatial relationships between components will govern the microbial community detected. For example, hallways, which act as viaducts for occupants between other rooms in a building, will have very different spatial and associated BE properties to a washroom. Kembel et al.[24] hypothesized that building form and organization, which are in turn dependent on the function of the space, are important determinants of the indoor microbiome. The authors used terminologies associated with network analysis (i.e., degree, betweenness, and connectedness) to describe the spatial characteristics of an indoor space, and associated changes in microbiome based on these spatial properties. Within spaces that share identical BE function (e.g., within office spaces), areas that were highly connected and closer to each other were also more similar in microbial communities. This was also seen in other BE microbiome studies comparing different buildings within various geographical ranges (see below). Recently, it has also been hypothesized that highly connected indoor rooms and spaces (serving different functions within a building) with minimal wall separation may also reduce microbial community divergence.[16]

6.08.6.3 Indoor Occupancy

Humans harbor a diverse community of microorganisms inside and outside the body. In occupied indoor settings, individuals emit as high as 10^6–10^7 bacteria and fungi per person per hour.[1] The influence of occupancy on the composition of airborne microbial community is associated with occupancy density and frequency and ventilation strategy, as discussed above. In addition to indoor air, occupancy has been shown to affect the diversity and structure of surface and dust microbial communities via different transmission mechanisms mediated by human behaviors and movements (see **Sections 6.08.5.2** and **6.08.5.3**). Similarly, pet ownership is also associated with increased diversity in the microbial community of household dust, which is likely to be the result of furred pets transporting exogenous microorganisms from outdoor environments into BE spaces.[7]

6.08.6.4 Vegetation

Like humans, plants are host to symbiotic microorganisms. In addition to a few plant and human pathogens, most of the microbial inhabitants tend to be beneficial and neutral. Although there are limited studies on this topic, indoor plants as a source of microorganisms have been recognized.[7] Based on our current knowledge, plants are a dispersal source of indoor environments in a way similar as human occupants, and plant-associated beneficial microorganisms can positively affect human health. Also, given that the outdoors act as important sources of the indoor microbiome, it is likely that both indoor and outdoor plants contribute to the indoor BE microbiome.[6]

6.08.6.5 Geography

The outdoor microbial community represents a diverse assemblage of microorganisms from multiple origins such as soils, plants, insect, animal feces, and water bodies.[6] Although a few species are ubiquitous, most microorganisms are more or less confined to certain localities. Since outdoor environments are a major source of microorganisms encountered indoors, the indoor microbial community is largely shaped by geography and location. Thus, distinct microbial community and/or indigenous species were identified for BEs from geographically different areas.[6] In addition to the geography pattern, the influence of landuse and urbanization degree on indoor microbiome is also evident. Dwellings in rural areas generally harbored abundant microorganisms of environmental origin due to the high exposure to green area (e.g., forest and farmland). Likewise, in urban regions, the composition of airborne bacterial community of outdoor BEs (e.g., parking space) was also affected by the nearby vegetation. However, urbanization generally reduced the indoor exposure to environmental microorganisms, and the indoor microbial community in more urbanized regions tended to be more homogenous.[25]

6.08.6.6 Climate

Climate plays a crucial role in regulating the richness and evenness of microbial communities in outdoor environments. Generally, warm temperatures in summer are associated with aerosolization of bacteria from soils, plants, and water sources, whereas these bacteria of environmental sources tend to be rare or absent in outdoor air during winter. Given the importance of outdoor microorganisms on indoor microbial community, significant pattern of seasonality was also detected indoors according to a previous time-series study (see **Section 6.08.5.3**). Although seasonal changes in meteorological factors can be buffered by the building envelops, the relative humidity of a single indoor space may vary largely within a short period of time due to human activities (e.g., shower in bathroom) and weather conditions (e.g., rainstorm). As indoor temperature is generally controlled at 15–30 °C, the growth rate of many microorganisms, especially fungal species, can be dramatically increased when relative humidity is elevated. BEs with serious moisture issue such as water-damaged homes are usually associated with the growth of visible molds, which can affect the composition of indoor airborne microbial community, and has been shown to induce health problems.[19] In addition to seasonal climatic changes, the BE microbiome is also subjected to occasional severe adverse weather conditions, resulting in alterations of the microbial communities that can persist for months after the onset of events.[2]

6.08.7 Virome and Archaeome in BEs

While the majority of the indoor microbiome is of bacterial and fungal origins, BEs also contain a viral community (the virome). Indoor environments may facilitate the transmission of viruses between occupants (see **Section 6.08.8**). Recent appreciation for understanding the indoor virome from a broader ecological perspective has applied metagenomic sequencing to survey viral communities in BEs. Given the small particle size and low abundance of viruses in indoor environments, the specialization and optimization of sampling and processing methods, such as the use of commercial kits specialized for extraction of viral genetic materials,[26] have been adopted. For example, to maximize yield of viral genetic material, Rosario et al.[26] performed a combination of sonication, physical disruption, filtration, and ultracentrifugation to facilitate viral release from filters, as well as the release of its genetic materials, and concentration of viral genetic material following extraction. In summary, thorough methodological optimizations are needed to collect sufficient viral genetic contents for its characterization in the BE.

To date, a diverse collection of DNA and RNA viruses have been detected across BEs that are thought to infect bacteria, fungi, humans, plants, and insects. For example, human skin-associated *Propionibacterium* phage has been detected across different BEs, while other viruses commonly detected in humans such as entero phages, papillomaviruses, polyomaviruses, and herpesviruses may be abundant and prevalent in BEs.[26] The same factors driving bacterial and fungal indoor microbial communities, such as ventilation mode and rate and human occupancy, may also affect indoor viral communities. Furthermore, viruses within indoor environments can come from pets, plants, and the outdoors.

Some of the remaining challenges in characterizing the indoor viral community involve the construction of a comprehensive viral database with high taxonomic resolution, as the majority of viral sequences currently detected in BEs cannot be classified to deeper taxonomic levels such as genus, species, and strains. Also, it is generally challenging to precisely identify the potential sources of viruses detected indoors, given their ability to travel long distances. In addition, care must be taken to describe the presence of human retroviruses in indoor settings, as some of these retroviral sequences may in fact originate from human host genome.

There is relatively limited information on the archaeal community (i.e., archaeome) of BEs, possibly due to the fact that the most common 16S rRNA gene region targeted in BE studies (i.e., V4) does not provide good coverage of archaeal species within the community. However, given that alternative primers have been shown to be more suitable for the characterization of archaea in other ecosystems,[27] archaeal taxa with potential human origins may be more readily detected in BEs in the future.

6.08.8 Risk of Pathogen Transmission Within BEs

One of the major incentives for a greater understanding of the indoor microbiome is to effectively prevent the transmission of diseases within such environments, therefore safeguarding occupant health. The importance of understanding BE designs and other factors in terms of pathogen transmission is perhaps best exemplified by the spread of the SARS virus within the Amoy Gardens residential complex in Hong Kong. In the work of Yu et al.,[28] the authors provided evidence that the SARS virus was likely originated from virus-contaminated soil stacks that have returned to bathrooms via dried water traps. Viral particles then may have entered the air shafts and moved upwards to enter different residences of the same unit within the building block. Viral particles that are now present in multiple levels within the same residential unit may also be transferred to neighboring units of the same building block, and also be transmitted to other building blocks within the complex through wind. Such a study emphasizes the importance of taking a multifaceted approach in understanding how pathogens are mobilized within and between indoor environments.

Examining the transmission routes of microorganisms within BEs, in hospitals for example, can aid in understanding how pathogens may spread within indoor environments, and how various building designs and anthropogenic factors shape pathogen spread. Following reports of respiratory conditions of lifeguards around a hospital pool area, Angenant et al.[9] were able to detect *Mycobacterium* sequences, among other potential respiratory pathogens, in the air directly above the indoor warm-water therapy pool, thereby providing possible mechanism of spread of mycobacterial pathogens from the pool to the lifeguards. More recently, using a fluorescence-based and particle-counting bioaerosol sensor, a hospital-based study demonstrated the potential for dispersion of particle-associated microorganisms from various textiles used in patient rooms across the hospital.[29] The authors reported that bacteria (some of which may be pathogens) presumably associated with hospital patients and personnel can be spread across multiple floors and be detected in textile holding rooms with minimal occupancy, suggesting that the spread of pathogens into a BE may not need to directly involve occupants. These studies also highlight the need for potential changes in building operation to prevent transmission of pathogens within BEs, such as increase of air circulation in the therapy pool environment[9] or additional personal protective equipment when handling hospital gowns and linens.[29]

A pre-requisite for host occupant infection is the survival of pathogens within the BE. Indeed, human pathogens grown in planktonic conditions showed reduced viability when inoculated onto BE surfaces, with a survival time on BE surfaces of less than a month.[30] However, organisms present on indoor surfaces may survive in the form of a biofilm, and laboratory assessments of viability of microorganisms within biofilms may be more appropriate. In the study of Marks et al.,[30] they inoculated and compared the viability of streptococci on plastic and hand surfaces following planktonic or biofilm growth in the laboratory. Biofilm cells remained viable on surfaces for extended periods of time compared to their planktonic counterparts, and biofilm cells were able to colonize the upper respiratory tracts of mice even after a month of being inoculated onto BE surfaces. Organisms directly collected from the indoor surfaces can also be cultivated, and can potentially resist antimicrobials, and subsequently transmit from one individual to others indirectly through survival on indoor surfaces.[2]

In addition to the spread of pathogens, building scientists and health professionals can also assess whether selective pressures imposed by occupants induce an effect on the BE microbiome (e.g., use of antimicrobial chemicals). Hartmann et al.[3] found that the abundance of antibiotic resistance genes was positively correlated with the application dose of specific antimicrobial chemicals within BEs. However, the authors were not able to correlate structural dissimilarities of the investigated indoor dust microbiomes with the applications of antimicrobials in the corresponding BEs. Additional studies will be required to provide a mechanistic explanation for the association between the concentrations of antimicrobial chemicals and relative abundances of resistance genes in the BEs.

Currently, we are only beginning to fully grasp the transmission, viability, virulence, and resistance properties of microorganisms within indoor environments, either by laboratory controlled experiments and/or characterization of microorganisms directly from field sampling. However, to further gain insights into how BEs directly or indirectly affect transmission of potential pathogens, a multidisciplinary approach is necessary by incorporating knowledge in building science, engineering, biology, and chemistry. Also, stemming from the growing appreciation that exposure to microbial metabolites and/or molecules may also affect occupant health, the integration of indoor microbiome data with data representing the exposure of the myriad chemicals we are exposed to daily will undoubtedly provide a more complete and integrated view of what occupants are exposed to in a BE.

6.08.9 Future Outlook

Previous observational and laboratory studies have been crucial toward our current understanding of the common microbial inhabitants of the different BE ecosystems, how experimental and analytical methods influence the BE microbiome data interpretation, the roles building designs and other environmental and anthropogenic factors play to shape the BE microbiome, the mechanisms of transmission of microorganisms and potential pathogens within and between the BE and its occupants, and the chemical molecules that are associated with indoor microorganisms. Recent advances in molecular microbiology technologies now enable us to obtain high-depth-resolution profiling of microbial communities in terms of composition, structure and function. For example, while previous clone library-based analyses provide functional potential profiles of the microbial community in and around BEs at limited depths, recent metagenomics analyses allow BE scientists to directly answer questions related to microbial virulence and resistance potentials within BEs, species- and strain-level classification of BE microorganisms and their potential sources, and various aspects of BE that may affect microbial functional profiles.[2,3-] Metagenomic sequencing also allows in-depth analysis of the microbiome consisting of organisms other than bacteria or fungi, such as the BE viral community. In addition, advances of sequencing technology platforms have pushed the development of new and improved bioinformatic tools that may allow for more meaningful analyses of the BE microbiome. For instance, the inherent low-biomass nature associated with some BEs have been met with benchmarking works[4,17] in order to maximize the biomass yield, while minimizing erroneous and contaminating sequences that may otherwise take up a large proportion of sequencing data.

Additional works are now being performed to focus on the viable and metabolically active portions of the BE microbiome, as DNA-based microbiome studies do not differentiate microorganisms that are viable and those that are not. While RNA-based studies looking at the viable microbial communities have been performed in other ecosystems, existing works attempting to understand the viable components of the BE microbiome are limited to fluorescent dye methods, which only allow the screening of non-active cells with a damaged cell membrane.[12] Recently, Gomez-Silvan et al.[12] assessed the viable portion of the residential air and surface microbiome via rRNA sequencing, which may be a more direct assessment of viability compared to methods based on cell membrane integrity. Adopting an optimized protocol for RNA isolation and preservation, the authors revealed that the viable components of the residential microbiome differ greatly from the total genetic pool of the same BE, and a sizable portion of the BE microbiome as determined by DNA methods may not represent viable organisms. However, due to differences in correlations between metabolic activity and rRNA levels,[12] careful interpretation of cellular activity using rRNA data is warranted. Nonetheless, additional BE studies focusing on viable components of the microbiome using rRNA sequencing is anticipated.

Environmental sustainability has become one of the most pressing scientific and social issues of the 21st century. Subsequently, there is a global rise in the construction and commission of environmentally friendly and energy-efficient buildings including green and zero-carbon buildings (ZCBs). These special buildings, with careful consideration of building use, geographical, and climatic factors, utilize specific innovative designs to minimize energy consumption while maximizing occupant comfort. Given our understanding of how building designs may affect the indoor microbiome, it is unclear whether the use of these energy-efficient BE designs may also affect the microbial communities of these buildings. Leung and colleagues reveal that a low-occupancy ZCB in Hong Kong presented air microbiome resembling that of other BE studies.[16] Specifically, the majority of the ZCB air microbiome consisted of taxa commonly associated with the outdoor environment, with a more modest influence from taxa associated with occupant skin. Although several studies reported similar relationships between occupant, environment, and BE regardless of green building certification, it is unknown whether these studies are representative of BEs around the world, as each BE is dependent on social, cultural, regulatory, and climatic considerations that are specific to the location of the BE.[16] Therefore, additional focus is required to gain insights into microbial exposures of occupants in energy-efficient buildings to provide a more comprehensive view of the energy-efficient building microbiome.

6.08.10 Conclusions

The need to safeguard the health and well-being of indoor occupants has been one of the driving forces for conducting BE microbiome research. Today, building scientists continue to characterize the indoor microbiomes of different BEs from both ecological and clinical standpoints. While cultivation studies have enlightened us in identifying some of the microorganisms that are present indoors, recent advances in culture-independent high-throughput sequencing technologies have greatly expanded our views of the BE microbiome. Also, the list of available analytic and bioinformatic tools for the characterization of the BE microbiome continues to expand, making this a truly exciting time for indoor microbiome research. Through collaborations between microbiologists, clinicians, engineers, and building scientists, we now have a drastically deeper understanding of various physical, temporal, environmental, anthropogenic, and other factors in shaping the microbial communities of indoor environments.

Future directions of the BE microbiome will undoubtedly aim at manipulating elements of indoor environments to enhance the health of occupants through alterations of the BE microbiome and creating a healthy living environment. Specifically, characterizing the non-pathogenic or beneficial microorganisms within the BE, and how they are affected by various factors, is crucial to ensure that these microbial members are retained within the BE and on occupants, thereby enhancing the health of indoor occupants. At the same time, by identifying indoor pathogens, their metabolites and pathogenic potentials, as well as how various factors affect the survival and transmission of pathogenic constituents of the microbiome, researchers can strive toward altering indoor conditions to either remove such pathogens or limit their transmission indoors.

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