

Seasonal Dynamics of the Airborne Bacterial Community and Selected Viruses in a Children's Daycare Center

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Introduction

Understanding the microbial community structure of the built environment is important because humans spend >90% of their time in indoor environments [1], and evidence is accumulating that both the human and environmental microbiomes shape human health [2–4]. Studies have characterized the microbiome of indoor air in residences, health care facilities, university classrooms, offices, restrooms, subways, and other settings [5–11], but less is known about children's daycare centers.

Over 8 million children attend daycare in organized facilities in the United States [12]. Children who attend daycare centers have a higher incidence of certain diseases and get sicker compared to children who do not attend daycare centers [13–18]. Thacker et al. [17] estimated that preschool-aged children who spend time in a daycare center are at 2–4 times greater risk for developing an infectious disease than are children cared for at home. Hurwitz et al. [19] reported an increased risk of respiratory illness in children attending daycare centers. Marbury et al. [20] found that daycare attendance is an important risk factor for lower respiratory illness and recurrent wheezing in young children. Effects extend to parents or guardians, who may lose an average of 13 workdays annually caring for children who are ill [21].

Models of the spread of diseases such as influenza in a community have shown that schools and childcare centers are hubs for transmission [22–27]. Many common respiratory illnesses have an airborne transmission route [28–31], so the air in a daycare center represents a unique opportunity for sampling certain pathogenic microbes. Respiratory syncytial virus (RSV), influenza, adenovirus, rhinovirus, rotavirus, and coxsackievirus A16 are commonly associated with disease outbreaks in daycare centers and can exist as bioaerosols [32–35]. For example, Yang et al. [36] found average concentrations of influenza A virus of 2×10^4 genome copies per m^3 of air in a daycare center in three samples that were positive for the virus; one sample was negative. Of course, the airborne microbiome encompasses all microorganisms, not just pathogenic ones. Prussin II et al. [37] reported total bacterial-like and viral-like bioaerosol concentrations in a daycare center to be 5.0×10^5 and 4.5×10^5 , respectively.

Prior studies have investigated the microbiome of daycare centers. A study of five daycare centers and five elementary schools in South Korea found that the airborne bacterial community reflected both humans and the outdoor environment [38], and there was no correlation between specific taxa and temperature and relative humidity. In a study of four daycare classrooms in the US, researchers swabbed toys and furniture and found sequences

that were related to *Pseudomonas* spp. and bacteria associated with humans, including some pathogens and opportunistic pathogens, and with wastewater sludge [39]. In these studies, sampling was limited to approximately two months [38] or did not include airborne microorganisms [39].

One question that has not yet been investigated, to our knowledge, is whether the airborne microbial community of a daycare center exhibits seasonal dynamics. The incidence of some diseases that occur commonly in children who attend daycare centers, such as hand, foot, and mouth disease (HFMD), influenza, and gastroenteritis caused by rotavirus, follows a seasonal pattern [35, 40–42]. Understanding how the airborne microbial community changes with season could provide insight into the epidemiology of seasonal infectious diseases [41]. Additionally, given the important role of viruses in respiratory disease outbreaks in daycare centers, studies examining viral bioaerosols have been especially limited, due to challenges in both sampling and data analysis [43, 44].

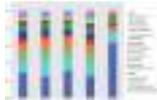
The objectives of this study are to determine the seasonal dynamics in the bacterial community structure and in the presence of selected pathogenic viruses in the air of a daycare center. We hypothesize that daycare centers harbor a unique and dynamic microbial community structure that is strongly influenced by occupancy. Additionally, we hypothesize that there are seasonal patterns in the community structure of airborne bacteria and the presence of selected pathogenic viruses. Our results provide novel information about the seasonal dynamics of airborne bacteria and viruses in a daycare center over a year-long period.

Bacterial Community

After trimming, sorting, and quality control, there were 138,047 sequences with a median sequence length of 251 bases for analysis. The unexposed filter did not produce a detectable 16S rRNA amplicon, and thus we assumed background bacterial contamination on the HVAC filters was negligible. We classified samples collected between 17 March–9 June 2014 as ‘Spring’, 9 June–2 September 2014 as ‘Summer’, 2 September–24 November 2014 as ‘Fall’, and 20 January–17 March 2014 and 24 November 2014–2 February 2015 as ‘Winter’. The sample collected between 23 December 2014–5 January 2015 corresponded to when the daycare center was closed, and thus we classified it as ‘Unoccupied’. We excluded the three samples collected between 20 January–3 February 2014, 3–17 February 2014, and 12–27 May 2014 from further analysis because the percentage of sequences that could not be assigned any taxonomy was 91%, 66%, and 67%, respectively. These percentages were much higher than the average unassigned rate of <3% across all other samples, indicating potential problems in the preparation and/or sequencing of these samples. An inherent limitation of the sequencing-based approach is that it does not discriminate between viable and non-viable organisms. Thus, the “community” that we describe is that defined by its genetic material.

Taxonomic analysis revealed that bacterial bioaerosols were diverse yet were dominated (greater than 60% of sequences in samples) by the classes Gammaproteobacteria, Alphaproteobacteria, Actinobacteria, Bacilli, and Betaproteobacteria (Fig 1). Additionally, the dominant bacterial phyla were Proteobacteria, followed by Firmicutes, Actinobacteria, and Bacteroidetes, with year-long relative abundance averages of 49%, 14%, 13%, and 9%, respectively. Three of these—Actinobacteria, Proteobacteria, and Firmicutes—also were the dominant phyla in a metagenomic study of airborne microorganisms in daycare centers and schools in South Korea [38]. A study of the bacterial composition of dust in daycare centers

in Finland showed that Gram-positive Bacilli and Actinomycetales (class Actinobacteria) dominated the airborne bacterial flora [50]. Prior studies of the microbiome in homes, office buildings, and classrooms have identified the same dominant phyla and classes [9, 51–53]. Given the current state of knowledge, the airborne microbiome of daycare centers appears to be similar to that of other indoor environments. It is possible that future tools may enable identification of unique features of the airborne microbiome of daycare centers, if they exist, but very carefully controlled studies would be required. A meta-analysis of 16 studies of the microbiome of the indoor environment found that technical variations between studies, such as geographic location and sample type, strongly affect comparisons of the microbial community [54].



[Fig 1](#)

Phylogenetic classifications to the class level by season and when the center was unoccupied.

The dominant phyla match the four that account for most of the typical human skin flora: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria [55]. The building where we collected our samples was occupied by ~100 children and their adult teachers at any given time. Humans shed 5×10^8 cells day⁻¹ [56]; thus human occupancy should have a dramatic impact on shaping the microbiome of the indoor environment. Qian et al. [10] measured bacterial bioaerosol emission rates of 3.7×10^7 bacterial genome copies per person-hour in a classroom. As in other studies of densely occupied environments [9, 57], our results suggest that the occupants of the daycare, and not the outdoor environment or other sources, are the main driver of the community structure of airborne bacteria.

[Fig 2](#) shows principal component analysis of weighted UniFrac distance for each sample, grouped by season except for the sole sample collected when the daycare center was closed and unoccupied during the winter holidays (white dot in [Fig 2](#)). The unoccupied sample exhibited a different community structure compared to samples collected when the daycare center was open. The relative abundances of Gammaproteobacteria and Flavobacteriia were higher in the unoccupied sample compared to the occupied samples, while the relative abundances of Actinobacteria, Bacilli, and Betaproteobacteria were lower ([Fig 1](#)). Weighted UniFrac distance was significantly different between occupied and unoccupied conditions ($p = 0.031$), but unweighted UniFrac distance was not ($p = 0.203$). Kembel et al. [6] observed a slight increase in the relative abundance of Gammaproteobacteria and a decrease in the relative abundances of Betaproteobacteria and Bacilli in outdoor air compared to indoor air of a mechanically ventilated building. These results suggest that the bacterial community is more influenced by the outdoor environment when the daycare center is closed, due to the decrease in human occupancy.



[Fig 2](#)

PCoA analysis of weighted UniFrac distance showing individual samples by season and one unoccupied sample.

According to principal component analysis, there were no apparent differences in the community structure of each sample between spring, summer, fall, and winter, as shown in [Fig 2](#). Alpha diversity, as measured by number of OTUs, showed no seasonal trend. A

more in-depth statistical analysis using ANOSIM (Table in [S2 Table](#)) suggested there to be a significant difference between seasons as measured using weighted UniFrac distance ($p < 0.05$), with the exception of the winter-spring pair. On the other hand, analysis using unweighted UniFrac distance demonstrated no significant difference between seasons ($p > 0.05$) with the exception of the winter-summer and spring-summer comparisons ($p < 0.05$). These results suggest OTUs within the daycare microbial community to be largely consistent across seasons, but with varying relative abundances.

Using ADONIS, we evaluated the correlation between temperature, relative humidity, and air velocity in the HVAC duct and weighted UniFrac beta diversity. For the variables of temperature and relative humidity, the correlation analysis indicated a significant correlation ($p = 0.02$ and 0.005 , respectively); however, the R^2 values were 0.067 and 0.08 , respectively, so the correlation explained only a small amount of variance. There was no significant correlation with air velocity in the duct ($p = 0.673$).

Previous work examining seasonal dynamics of microbes in indoor air has focused mainly on the concentrations of viable bacteria and fungi. Reponen et al. [\[58\]](#) showed a seasonal effect on the concentration of viable fungi in household air, while Moschandreas et al. [\[59\]](#) suggested that summer and fall produce the highest concentrations of cultivable bacteria in the air of homes. Due to the seasonality of both ambient conditions and certain diseases [\[41\]](#), we expected to observe seasonal differences in the community structure, but pathogens account for a small fraction of total bacteria. We also expected to observe seasonal differences because Bowers et al. [\[60\]](#) showed that the community structure in outdoor air is strongly affected by season. However, it appears that building occupants strongly influenced the microbiome of the daycare center's air, obscuring any seasonality that might have existed in bacteria originating from outdoor air. In studying the seasonal dynamics of bacteria in dust collected from office buildings, Rintala et al. [\[53\]](#) also did not find clear differences in microbial community structure between seasons.

These results suggest that human occupancy, and the shedding of skin cells, is a major factor in determining the microbial community structure of airborne bacteria; and in heavily occupied environments such as daycare centers, schools, and offices, human occupancy has greater influence than does the outdoor environment in shaping the airborne microbiome. It is possible that in a very well-ventilated and low-occupancy environment, seasonal differences in the community structure of airborne microorganisms will be observed. This topic is an excellent avenue for future research.

3.2 Dynamics of Selected Viruses

Using PCR, we determined the presence or absence of adenovirus, enterovirus-71, IAV, RSV, rhinovirus, and rotavirus in samples based on whether an amplicon was produced at the same size as a positive control. [Table 3](#) shows results for adenovirus, RSV, and rotavirus, where a plus sign indicates the virus was present based on a PCR positive result for the sampling period. We were not able to reach definite conclusions for enterovirus-71, IAV, or rhinovirus because PCR results indicated contamination on the HVAC filters for these viruses. Although two of our negative controls, the PCR water blank and the extraction kit blank, did not produce any PCR products, the unexposed filter did produce an amplicon at the correct size for the region being amplified. This suggests the possibility that the HVAC filters were contaminated with enterovirus-71, IAV, and rhinovirus and that the contamination was present on the unexposed filter and did not originate from the PCR reaction or extraction kit. Upon further investigation, we learned that the HVAC filters were not machine-produced but

were mounted in the cardboard frame by hand, thus providing a possible avenue for contamination with human-associated microbes. It is critical that any future studies using PCR on bioaerosols collected from HVAC and other types of filters attempt to remove pre-existing nucleic acid by subjecting them to ultraviolet radiation, bleach, or some other decontamination method immediately prior to sampling.

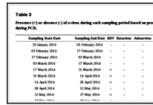


Table 3

Presence (+) or absence (-) of a virus during each sampling period based on production of amplicon during PCR.

We detected RSV during two extended periods: 17 March 2014–21 July 2014 and 27 October 2014–2 February 2015 (Table 3). RSV is a lower respiratory tract viral pathogen in infants and young children that causes common-cold-like symptoms [61]. Lindsley et al. [62] showed that RSV occurred in aerosols small enough to remain airborne for an extended time, be transmitted via the airborne route, and cause infection. RSV is a seasonal virus that typically first appears in the northern hemisphere in November with an epidemic period lasting approximately four months [63]. In the southern hemisphere RSV typically first begins to cause infection in March or April. Our study did not collect any human information (e.g., health, travel, etc.), so we cannot confirm whether there was an out-of-season outbreak at the daycare center in the spring and early summer. Seasonality does appear to play a role in shaping the presence of RSV in the air of a daycare center.

We did not detect adenovirus and rotavirus at any time throughout the year (Table 3). This finding was contrary to expectations for adenovirus, as Fairchok et al. [32] suggested the virus causes 22% of respiratory tract infections in daycare centers. Airborne transmission of adenovirus in the environment has been demonstrated [64, 65], and the virus has been found in the air of a pediatric department of a hospital [66]. Further studies are needed to explain why the virus was not detected. Rotavirus causes a significant amount of diarrheal diseases in daycare centers [67, 68], and Dennehy et al. [69] detected rotavirus in 75% of air samples collected in hospital rooms with rotavirus-infected patients, even though the virus is shed in feces and is primarily transmitted via the fecal-oral route through contaminated hands, surfaces, and fomites [70, 71]. The daycare center we sampled has very strict policies regarding sanitization after diaper changes, washing hands frequently, and immediately sending home children with diarrhea, so the opportunities for aerosolization may have been minimal. Babies and toddlers do not use toilets, which can aerosolize fecal pathogens [72–74]; soiled diapers go directly into the trash. Additionally, the rotavirus vaccine became routine in 2006 in the United States [75, 76]. The vaccine is highly effective, reducing hospitalizations and emergency department visits by more than 80%.

We did not collect any human-subject related information (e.g., health records, travel information, etc.); however, in the future it would be valuable to correlate disease outbreaks in a daycare center with the airborne microbial community. Additionally, future studies should investigate microbial communities in the air, on surfaces, and in/on humans simultaneously at a daycare center; the continuing decrease in sequencing costs would make such a study feasible. Initially, the goal of our study was to examine viral communities using metagenomics; however, we were unable to obtain useful sequence data, most likely due to sampling in a limited-biomass environment (i.e., the air compared to water or soil). Using metagenomics to examine airborne viral communities has remained elusive due to challenges in both sampling and data analysis [43, 44].

Conclusions

We examined the airborne bacterial community and specific viruses collected on HVAC filters in a daycare center over a full year. Bacteria associated with human skin dominated the samples. The community structure differed when the center was occupied v. unoccupied, supporting the idea that human occupancy plays a critical role in shaping the indoor air microbiome. Seasonal dynamics were not evident in the bacterial community structure. Airborne RSV was present during wintertime, matching the typical seasonal pattern of the disease; RSV was also present in the spring through early summer. We did not detect adenovirus or rotavirus at any time. As we found HVAC filters to be contaminated with certain virus species, we urge future researchers to attempt to sterilize the filters prior to sampling.

Acknowledgments

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