Title: Microbial Sharing between Pediatric Patients and Therapy Animals during Hospital Animal-Assisted Intervention Programs

Authors:
Kathryn R. Dalton¹, Kathy Ruble², Laurel E. Redding³, Daniel O. Morris⁴, Noel T. Mueller⁵, Roland J. Thorpe Jr.⁶, Jacqueline Agnew¹, Karen C. Carroll⁷, Paul J. Planet⁸, Ronald C. Rubenstein⁹, Allen R. Chen², Elizabeth A. Grice¹⁰, and Meghan F. Davis¹,¹¹

Author affiliations:
¹ Johns Hopkins University Bloomberg School of Public Health, Department of Environmental Health and Engineering, Baltimore Maryland
² Johns Hopkins University School of Medicine, Departments of Oncology and Pediatrics, Baltimore Maryland
³ University of Pennsylvania School of Veterinary Medicine, Department of Clinical Studies, Kennett Square, Pennsylvania
⁴ University of Pennsylvania School of Veterinary Medicine, Department of Clinical Sciences & Advanced Medicine, Philadelphia Pennsylvania
⁵ Johns Hopkins University Bloomberg School of Public Health, Department of Epidemiology, Baltimore Maryland
⁶ Johns Hopkins University Bloomberg School of Public Health, Department of Health, Behavior and Society, Baltimore Maryland
⁷ Johns Hopkins University School of Medicine, Department of Pathology, Division of Medical Microbiology, Baltimore Maryland
⁸ University of Pennsylvania Perelman School of Medicine, Department of Pediatrics, Philadelphia Pennsylvania
⁹ Washington University in St. Louis School of Medicine, Department of Pediatrics, Division of Allergy and Pulmonary Medicine, St. Louis Missouri
¹⁰ University of Pennsylvania Perelman School of Medicine, Department of Dermatology, Philadelphia Pennsylvania
¹¹ Johns Hopkins Medicine, Department of Molecular and Comparative Pathobiology, Baltimore Maryland

Funding Source:
Funding for this project was supported by the Morris Animal Foundation [D15CA-802] and the National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development [5R01HD097692-02]. Funding for KRD is provided by a grant from the U.S. Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health to the Johns Hopkins Education and Research Center for Occupational Safety and

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Health [T42 OH0008428], and the AKC Canine Health Foundation Clinician-Scientist Fellowship [02525-E]. MFD was supported by the National Institutes of Health (K01OD019918).

Acknowledgements:
The authors would like to thank Alexandra DeLone, Pamela Frankenfield, Destiny Walker, Shanna Ludwig, Janice Jaskulski, Kaitlin Waite, Sabrina Waugh, Zoë Johnson, Erin Beasley, Christopher Nelson, Andrea Christ, Kristopher Spicer, Katie Sabella, MaryJane Schroeder, Amy Dickman, Frederic Askin, Amy Wernecke, Aaron Milstone, and Clare Rock for their assistance, cooperation, and guidance.

We would also thank the teams at Eurofins Scientific and the Children’s Hospital of Philadelphia (CHOP) Microbiome Center, High-Throughput Sequencing Core for technical assistance.

Data Availability:
The dataset supporting the conclusions of this article is available in the NCBI Sequence Read Archive (SRA) database under BioProject PRJNA695069, BioSample SAM17600695. Unix and R code used for analysis can be found under KRD’s Github repository:
https://github.com/kathryndalton/AAT_pilot_analysis.
Abstract

Background: Microbial sharing between humans and animals has been demonstrated in a variety of settings. However, the extent of microbial sharing that occurs within the healthcare setting during animal-assisted intervention programs, a validated and valuable part of holistic patient wellness, is unknown. Understanding microbial transmission between patients and therapy animals can provide important insights into potential health benefits for patients, in addition to addressing concerns regarding potential pathogen transmission that limits program utilization. This study evaluated the potential for microbial sharing between pediatric patients and therapy dogs, and tested whether patient-dog contact level and a dog decolonization protocol modified this sharing.

Methods and Results: Patients, therapy animals, and the hospital environment were sampled before and after every group therapy session and samples underwent 16S rRNA sequencing to characterize microbial communities. Both patients and animals experienced changes in the relative abundance and overall diversity of their nasal microbiome, suggesting that exchange of microorganisms had occurred. Increased contact was associated with greater sharing between patients and therapy animals, as well as between patients. A topical chlorhexidine-based dog decolonization intervention was associated with decreased microbial sharing between therapy dogs and patients, particularly from the removal of rarer microbiota from the dog, but did not significantly affect sharing between patients.

Conclusion: These data suggest that the therapy animal is both a potential source of and a vehicle for the transfer of microorganisms to patients but not necessarily the only source. The relative contribution of other potential sources (e.g., other patients, the hospital environment) should be further explored to determine their relative importance.
Introduction

Animal-Assisted Intervention (AAI) therapy, the use of animals as an alternative or complementary treatment, can improve the physical, mental and social functions of patients within the healthcare setting. AAI has been widely implemented in a range of physio-social conditions in various settings in healthcare facilities and is increasingly popular, especially for pediatric patients. The most commonly reported patient benefits include a reduction in patients’ requirement for pain medication, enhanced socialization, and reduced stress and anxiety (Bert et al., 2016; Charry-Sanchez et al., 2018b, 2018a; Kamioka et al., 2014; Waite et al., 2018).

Conversely, the potential risks of incorporating animals into a hospital setting, where patients with decreased immune function are treated, must be considered. Nosocomial transmission of infectious disease agents, such as methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is a serious problem exacerbated by close contact and antimicrobial selective pressure inherent to healthcare settings, and we were concerned that therapy animals may serve as mechanical vectors of transmission. While it is clear that therapy dogs can carry common hospital-associated pathogens (Boyle et al., 2019; Dalton et al., 2020; Lefebvre et al., 2009), evidence is lacking on whether dogs transmit these microbiota to patients.

Microbes, including pathogens, function in the context of a more global microbial community, and other non-pathogenic microbiota may similarly be transmitted during these AAI sessions. Specifically, dogs have unique compositions of their nasal, dermal, and gastrointestinal microbial communities (Hoffmann et al., 2014; Oh et al., 2015; Swanson et al., 2011), which, compared to humans, could result in a distinct ability to acquire, carry, and/or spread hospital-associated pathogens. These distinct microbial communities could also uniquely influence the microbial composition of individuals that interact with the dogs, in a way that is fundamentally different than contact with other people or objects in the environment. This circumstance is best illustrated by data demonstrating the microbial shifts in humans resulting from pet ownership;
pet owners often have more diverse microbial compositions that are more frequently shared between them (Misic et al., 2015; Song et al., 2013). Early life pet ownership is associated with decreased incidence of immune dysfunction, and exposure to diverse microbes from farm environments, including animals, is protective against the development of asthma in children (Azad et al., 2013; Fall et al., 2015; Stein et al., 2016; Tun et al., 2017). However, these studies focus on chronic exposure from living with animals and pets. It is uncertain if these same microbial shifts will occur with transient exposure of patients to a therapy animal, which in our setting was often less than one hour.

This study aimed to explore the potential for microbial sharing between pediatric patients, therapy animals, and the hospital environment during animal-assisted intervention programs. We hypothesized that therapy dogs could serve as intermediary mechanical vectors in the transmission of microbes between the hospital environment and patients, and interaction with the therapy animal would increase patients’ risk of microbial exposure (Figure 1). We further examined whether the level of contact between patients and therapy dogs modifies this microbial sharing. This study used a topical antiseptic treatment on the therapy animal as a targeted intervention to mitigate potential risks from exposure to infectious agents to patients participating in AAI. We secondarily hypothesized that this topical disinfectant, aimed at decreasing the bacterial colonization in the therapy animal, would have downstream effects on microbial composition in patients. Improving our understanding of microbial dynamics that occur during an AAI session will contribute to our knowledge base regarding human-animal microbial exchange research in a novel setting and have practical implications to AAI program implementation.
Methods

I. Experimental Design and Sample Collection

This study was conducted at a pediatric oncology outpatient unit in a mid-Atlantic hospital between July 2016 and May 2017. The study protocol was approved by all applicable institutional review boards, institutional animal care and use committees, and scientific review committees prior to data collection. All therapy dog handlers and patients’ parents provided written consent to participate in the study and approved having the findings published. The therapy dog and handler were scheduled for one hour in a shared space, during which multiple patients interacted with the dog at the same time. Microbial samples were collected from the nasal mucosa of pediatric patients and therapy dogs with a sterile flocked swab (Puritan, Guilford, ME, USA) before and after the AAI visit, as well as the shared floor space with a vacuum dust filter (Ludwig et al., 2017). Trained research staff performed all sample collection. During the visit, we observed interactions of the study participants with the dog, recording the total duration and frequencies of certain behaviors (petting, hugging, etc.). Blank sterile flocked swabs were collected at every visit as a negative control. Sample swabs were stored at -80°C until processing.

The therapy dog team, consisting of the dog and its handler, completed two observational control visits abiding by established hospital protocol, then crossed-over to two intervention visits with modifications to the hospital therapy dog protocol, as shown in Supplemental Figure 1. Prior to the first intervention visit, the handler was given a 4% chlorhexidine-based veterinary prescription shampoo (DUOXO Ceva, Libourne, France) to use 24 hours before the study visit. During the therapy visit, the dog was wiped down along the head and back, the “petting zone”, with 3% chlorhexidine wet cloths (DUOXO Ceva, Libourne, France) every 5 to 10 minutes.

II. Laboratory Processing
II.a. 16S rRNA Gene Amplification and Sequencing

Sample swabs and vacuum filter dust were thawed prior to DNA extraction. DNA sequencing was performed as previously described (Misic et al., 2015); see also Supplemental Methods for additional details. For each set of extractions, one blank swab exposed to laboratory air was processed as a negative laboratory control. Prior to sequencing, the total DNA concentration was obtained from a Qubit instrument, and the 16S rRNA gene copies per unit DNA were evaluated using quantitative PCR. The V1-3 region of the 16S rRNA gene was amplified using barcoded primers (27F, 534R) for the Illumina platform as previously described (Fadrosh et al., 2014). Sequencing was performed on the MiSeq instrument (Illumina, San Diego, CA) using 300 base paired-end chemistry at the University of Pennsylvania Next Generation Sequencing Core. Microbial Mock Communities B (Even-Low v5.1, BEI Resources, NIAID NIH HMP) were amplified and sequenced as positive controls.

II.b. Bioinformatics and Quality Control

QIIMEv2.7 was used for paired-end read assembly and quality filtering for the sequences from all samples (Bolyen et al., 2019). The DADA2 plug-in for QIIME2.7 was used to remove chimeric sequences and sequences greater than 300bp in length, and cluster sequences into amplicon sequence variants (ASVs) (Callahan et al., 2016). ASVs were matched to phylogeny using mafft program for multiple masked sequence alignment (Katoh et al., 2002) and FastTree to generate a phylogenetic tree from the masked alignment (Price et al., 2010). Taxonomy assignment used a Naive-Bayes classifier (Wang et al., 2007) that was trained on our dataset (trimmed to 300bp and matched to our primers), applying Greengenes13.8 99% OTU match (McDonald et al., 2012). Taxonomic classification was confirmed by comparing the identification of the known Mock Community samples. For quality control purposes, suspected contaminants were identified and removed from the resulting feature table using the ‘decontam’ R package,
based on the prevalence of taxa in the negative controls and the frequency of taxa as a function of the total DNA concentration and the 16S rRNA copies from qPCR (Davis et al., 2018). Contaminants were identified independently at each processing step (field sampling, DNA extraction, and sequencing) and were sequentially removed. Information on the sequencing library and quality control measures can be found in Supplemental Tables 1A&B.

III. Statistical Analysis

Statistical analysis was performed in RStudio v1.1.423 (R Development Core Team, 2010). To maintain the maximum number of samples for comparison, the sequencing data was not rarefied for statistical analysis (McMurdie & Holmes, 2013; Willis, 2019). Taxa tables, and matching phylogeny and taxonomy, were analyzed using the phylseq pipeline to calculate alpha and beta diversity metrics (McMurdie & Holmes, 2013). The primary analysis was the change in microbial composition comparing pre and post visit overall and by host (human and dog), then stratifying by contact level and visit type (control versus intervention). Differential abundance of specific taxa between groups were analyzed using DESeq2 (Love et al., 2014). The Kruskal-Wallis nonparametric one-way analysis of variance test examined differential alpha diversity between all groups, and the Wilcoxon rank-sum test was used for pair-wise comparisons between groups; both tests were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) correction. To test which factors were most important in determining microbial composition, analyses were performed using the non-parametric permutational multivariate analysis of variance (PERMANOVA) with weighted and unweighted UniFrac distance metrics.
Results

I. Study Population and Samples

A total of four dogs were studied over 13 AAI visits (2-4 visits per dog team), with 5 (38%) being intervention study visits. Forty-five unique pediatric oncology subjects enrolled in the study (Table 1), with a mean age of 11.7 years old (SD 4.7). Four participants re-enrolled in the study, resulting in data from 49 study participants. Each therapy visit had a mean of 3.8 participants (SD 1.4, range 2-6). Thirty-nine participants (79.6%) reported having a pet at home, with 30 (61.2%) having a dog.

Individual contact behaviors and total patient-dog interaction times are presented in Supplemental Table 2. The frequency of key behaviors and total time spent with the therapy dog were aggregated to create an ordinal contact score. The median contact score was used as a threshold to create a binary contact level of “High” or “Low” contact. Fifty one percent of patients were classified as “High” contact, and this was evenly distributed across control and intervention visits.

A total of 129 sample swabs were collected for microbial analysis (Table 1). An additional 33 samples were processed for microbiome quality control. Swab samples were not collected from 8 participants either due to the patient’s fear of the swabbing process or scheduling conflicts. Two subjects did not have pre-visit culture swabs collected, and three subjects did not have post-visit swabs collected.

II. Relative Abundance

The abundance of microorganisms differed across both host and sample location; Figure 2A shows the percent relative abundance of the top 25 most abundant genera. Certain bacterial species had significantly different abundance when comparing across sites, including...
Staphylococcus species in the nasal samples of both pediatric subjects and dogs (Figure 2). Subject and dog nasal samples had similar microbial compositions, with Staphylococcus species being dominant, but dog nasal samples had a greater abundance of Moraxella compared to the subjects’ greater abundance of Streptococcus. These data are summarized in Supplemental Table 3.

The degree of alteration of patients’ microbial communities varied with contact level and visit type (Figures 2B&C). Within control visits, subjects with low contact had a higher abundance of Streptococcus species after the visits compared to before. In contrast, there was no difference in the abundance of any genera between pre- or post-visit samples in high-contact subjects. Within the intervention visits, both high and low contact subjects had greater abundance of Streptococcus species before the visit and greater abundance of Staphylococcus species after the visit, specifically S. epidermidis and not S. aureus (Supplemental Figure 2).

III. Alpha Diversity

Alpha rarefaction curves are presented in Supplemental Figure 3. Alpha diversity significantly differed between hosts (humans versus dogs versus environment), as measured by the observed total taxa, Shannon, and Faith’s Phylogenetic metrics (Wilcoxon rank-sum test p<0.001). This was a consistent observation when stratifying by pre or post visit, and by control or intervention visit type.

When examining individual level changes in alpha diversity that occur during a therapy visit, in high-contact subjects there was an overall increase in within-sample diversity during control visits, and an overall decrease during intervention visits; either no difference or the opposite difference occurred in low-contact subjects (Figures 3A and 3B). The change in alpha diversity between pre- and post-visit samples was significantly different in control versus intervention visits in high contact patients when measured with Faith’s metric (Kruskal Wallis p<0.05), but
not the Shannon metric or observed total taxa. A similar significant effect could be seen in therapy dog samples when using Faith’s metric (Kruskal Wallis p<0.01), with an overall increase in alpha diversity following control visits, and a decrease following intervention visits (Figure 3.D-F).

IV. Beta Diversity

IV.a. Beta Diversity Distribution

Supplemental Figure 4 shows the overall distribution of samples in principal coordinate analysis plots for both unweighted and weighted UniFrac beta diversity metrics, by hosts (pediatric subjects, dog or hospital environment), site, and pre- and post-visit status. Loose clustering was observed by host and sample site, but not by sample timing (pre vs. post). Clustering was also not observed by individual subject or visit date. Overall the axes accounted for a maximum of 7.8% variation in unweighted UniFrac and 33.5% variation in weighted UniFrac.

IV.b. Beta Diversity Distance

Pediatric subjects were more similar to other subjects after the visits, as evidenced by their reduced microbial composition beta diversity distance (PERMANOVA pre vs. post FDR-p<0.001). Patients were also more similar to therapy dogs after the visits (PERMANOVA pre vs. post FDR-p<0.001). See example calculations in Supplemental Figure 5, and results in Supplemental Figure 6 and Supplemental Table 4.

Subjects with high contact were more similar to other subjects (Figure 4A) and to the therapy dog (Figure 4C) after the visits, than to low contact subjects (unweighted UniFrac metric PERMANOVA FDR-p=0.0001-0.0003). The same pattern was observed in both control and intervention visits. Using a weighted UniFrac metric, high contact subjects were more similar to
other subjects in control visits (p=0.0005), but not in intervention visit (Figure 4B). The reverse trend was observed between patients and the dog, with both high and low contact patients more similar in microbial composition to the therapy dog following intervention visits (p=0.0001, 0.0005) but not control visits (Figure 4D).
**Discussion**

This study explored microbial transmission among pediatric oncology subjects and therapy animals in a hospital-based AAI program. This study is the first to report on sampling patients, therapy animals, and the hospital environment before and after a group AAI session, and the first to explore microbial community dynamics in this setting. Our data suggest that microbial sharing occurred during the AAI sessions, as microbial compositions of subjects were altered, both in overall diversity levels and relative abundance of specific taxa. We further explored the effect of contact level between patients and therapy dogs on the alteration of nasal microbial communities following visits, and logically found that higher contact was associated with increased sharing between subjects and therapy animals, and among subjects. Finally, we determined that an antiseptic decolonization intervention targeted to the therapy dog modifies the association between contact level and microbial sharing between therapy animals and subjects, and between subjects as well.

**I. Distinct Microbial Profiles and Shifts in Patients and Therapy Dogs**

Patients, therapy dogs, and the hospital environment had distinct microbial communities, as evidenced by differences in the relative abundance of key species, differences in alpha diversity, and unique clustering of microbial composition in beta diversity. Human and dog nasal sites tended to be dominated by a few taxa at relatively high abundance (namely *Staphylococcus*, *Streptococcus*, and *Moraxella*), and had distinct beta diversity clusters on PCoA plots. These data are confirmed from other studies that have evaluated the microbiome of human skin and nasal samples (Brooks 2017, Lax 2015, Adams 2015, Oberauner 2013).

We observed microbial community shifts in pediatric subjects and therapy dogs during an AAI therapy session. This was demonstrated by the increase in within-sample alpha diversity levels in subjects and dogs, more similar microbial compositions between groups following the visits,
and changes in the relative abundance of certain taxa, specifically *Staphylococcus*. Beta diversity distance, represented by both unweighted and weighted UniFrac metric, was calculated as the difference in beta diversity between subject samples and between subject and dog samples. We then used these metrics as a proxy for the degree of microbial sharing between these hosts, as these metrics best indicated shifts in the microbial community structure that occurred during the visits. From these data, we found that subjects had more similar nasal microbial structure as other subjects and therapy dogs after the therapy visit compared to before, suggesting that sharing of microbiota occurred. Such sharing has been demonstrated in other human-animal microbiome studies, particularly those that evaluated pets in the home (Song 2013, Misic 2015).

**II. Closer Contact Between the Patient and Therapy Dog Increased Microbial Sharing**

Our data also suggest that patient-dog contact level modifies microbial sharing between subjects and therapy dogs, as well as between subjects. While contact level was primarily an indicator of the degree of interaction between a subject and a therapy animal, by extension it can also reflect the degree of contact that occurs between a patient and the hospital environment, other patients, and other aspects of the therapy visits (model shown in Figure 1). In other words, a subject with a high contact score will have higher contact with the therapy dog, as well as with other patients and individuals, including the therapy dog handler, and with the hospital environment. Thus, it is logical that high contact with the therapy animal suggests higher contact with the more general environment, and this was reflected in our data as being positively associated with increased microbial sharing.

Our data suggest that high-contact patients with more interaction with various aspects of the therapy programs shared more microbes both with other patients and with the therapy dogs. This was demonstrated by an increase in within-sample alpha diversity and more similar beta
composition between samples. Interestingly, there were differences using phylogenetically weighted versus unweighted metrics. Faith’s Phylogenetic alpha diversity changes were greater than Shannon alpha diversity, suggesting that more phylogenetically distinct microbiota are driving the increased alpha diversity in subject samples. Our unweighted UniFrac distance appeared to show stronger microbial sharing between high contact patients and therapy dogs, and among high contact patients, while the phylogenetically weighted UniFrac distance appeared to show significant sharing of rare taxa among high-contact subjects, but not between humans and dogs.

Taken together, these data suggest that bacteria are shared among humans, and between humans and dogs, in the AAI setting, but rare bacteria are less commonly shared between humans and dogs. Our PCoA distributions and relative abundance results, in addition to previous studies on pet dogs (Davis, 2016; Oh et al., 2015; Ross et al., 2018; Song et al., 2013), have shown that dogs have distinct microbial communities compared to humans. These differences could possibly drive the differences we observed in weighted beta metrics comparing subject-to-dog composition difference to subject-to-subject composition difference. These data therefore support the hypothesis that dogs can serve as intermediary vectors in the spread of human-origin common microbiota between patients, but may not be sharing their own unique microbiota with patients. However, significant sharing of rare taxa occurred among subjects in the AAI setting. These additional data suggest that the therapy animal is only one potential pathway by which microbes can be transmitted during these group AAI therapy sessions, with other pathways shown in Figure 1 potentially being more influential.

**III. Canine Decolonization Intervention Modified Microbial Sharing**

We tested a novel application of a topical chlorhexidine to therapy dogs and assessed how this canine decolonization intervention influenced microbial sharing between patients and dogs and
among patients. Our data preliminarily demonstrate that the dog microbial decolonization intervention modifies the observed relationship between contact level and microbial sharing. The decolonization intervention appeared to have influenced more phylogenetically distinct, rare taxa, as different outcomes were obtained using phylogenetically weighted versus phylogenetically unweighted diversity models. Within the intervention visits, microbial sharing of common taxa was still observed among subjects and between subjects and therapy dogs, as evaluated by the unweighted UniFrac distances. However, unlike in control visits, the weighted UniFrac distances suggest that rare taxa were not shared among subjects. Thus, the intervention appears to have blocked the sharing of rare phylogenetically diverse taxa between humans. High-contact subjects had more significantly decreased alpha diversity levels following intervention visits than in control visits, indicating that our canine-centered decolonization had indirect effects on the microbial diversity levels of human subject samples. These data are consistent with previous data on the effects of the hospital built-environment microbiome on patient microbial composition, and particularly those data regarding the influence of environmental cleaning regimens on patient microbiota (Brooks 2014, Ramos 2015, Dalton 2020).

Interestingly, following intervention visits, both high and low contact patients appeared to have more similar microbial compositions as therapy dogs, using the phylogenetically weighted UniFrac metric. This contrasts to control visits where subjects of both contact levels had less similar microbial communities to dogs. This difference is explained less by microbial sharing, as the effect of the disinfectant intervention on the dog’s microbiota. The decolonization selectively removes unique dog taxa from the dog itself, perhaps more easily allowing recolonization with the microbial community of the subjects.

The intervention was also associated with changes in the abundance of specific taxa. High-contact patients had higher relative abundance of staphylococcal species following intervention
visits compared to high contact patients following control visits. This change was primarily

driven by \textit{S. epidermidis}, a predominant human nasal and skin commensal, rather than \textit{S. aureus} that can be more pathogenic. Because this metric compares relative rather than

absolute abundance within each sample group, it is not surprising that human commensals are

of greater relative abundance in intervention visits than control visits, since the subjects were

exposed to fewer taxa from the therapy dog.

Overall, while the intervention influenced microbial composition, diversity levels, and sharing

among humans, it primarily exerted these effects by modulating the therapy dog’s microbial

composition. If the therapy dog was the only or primary source of microbes that were transferred
to patients during AAI sessions, we would expect to see reduced sharing of both common and

rare taxa between patients and dogs following intervention visits when the therapy dog pathway

is blocked. Since we only see this pattern with phylogenetically distinct, rare taxa, not common
taxa, it appears more likely that the therapy dog serves as an intermediary point of microbial

sharing, rather than a source of microbes. Thus, the dog is only one of many possible pathways

of microbial sharing (\textbf{Figure 1}), and these other pathways may contribute more to microbial

changes seen in subjects attending AAI visits.

\textbf{IV. Strengths, Limitations, and Future Directions}

While designed as a pilot study to assess feasibility, this study expressly targets microbial

transmission that occurs during hospital AAI programs and the first to report on sampling

multiple components before and after each visit. As such, these data provide a critical

foundation for larger studies in this area. Previous studies have focused exclusively on carriage

in the therapy animal or have assessed aggregated rates of infection diagnosis in departments

with or without AAI programs (Dalton et al., 2020). By sampling multiple components—the

patients, the therapy animals, and the hospital environment—we can begin to elucidate
exposure pathways from these individual data points. In addition to our novel sampling strategy, this is the first study to assess the effect of patient-dog contact on microbial sharing. Human-animal contact level has been previously described as a risk factor for exposure and acquisition of pathogens in the case of pet ownership (Morris et al., 2012; Rodrigues et al., 2018). However, it was unknown if the same positive association would occur with the transient contact between patients and therapy animals. This study also benefits from the novel deployment of an established canine decolonization procedure, adapted from veterinary clinical protocols for canine patients with dermatopathologies. The intervention appeared to limit the spread of the therapy dog’s own unique microbiota to patients, and also reduced the therapy dog’s role as an intermediary vector in the spread of microorganisms between patients or other individuals and the hospital environment.

This study does have practical limitations. As a feasibility study in preparation for a larger infection control trial, including the implementation of the decolonization intervention, it is limited by small sample size, particularly when considering the number of unique dogs. While our sampling was fairly extensive, sampling other sites, both on the subjects and in the hospital environment, as well as other individuals, such as healthcare workers and the handlers, may have provided additional data that supported alternative hypotheses. Multiple pathways depicted in Figure 1 are, in fact, quite challenging to examine, and blanket statements inferring directionality of transmission from therapy dogs to patients or vice-versa should be taken with appropriate caution. Finally, this experiment assessed microbial exposure and composition at one time point. Our data do not address if the changes observed during the visit will persist and, if so, for how long. These data also do not support claims regarding the health outcomes related to these microbial community shifts, particularly related to the exposure of potentially pathogenic microorganisms and to rare taxa from the therapy dog.
Future work on this topic will expand to studying AAI sessions that involve only one child per
dog, thus providing more controlled insight into potential microbial transmission pathways, and
increase the generalizability of findings to other situations. Studies that sample within different
hospital departments with varying compositions of patients, and various hospitals will also be
required to increased generalizability. Lastly, longitudinal studies are required to explore the
temporal stability of these microbial shifts observed in patients and determine if it leads to
clinically significant outcomes. Such longitudinal studies are especially important when
considering the exposure to rare dog taxa, given that early-life exposure to pets is associated
with decreased incidence of allergic and atopic diseases in children (Havstad et al., 2011;
Mandhane et al., 2009), and having a diverse microbiome is protective against numerous health
outcomes and can be protective against colonization from pathogens (Grice & Segre, 2011;
Naik et al., 2012). If such data suggest that exposure to therapy animals, even briefly during AAI
programs, can benefit microbial diversity and microbial community resilience over a longer-term,
this will be a previously undescribed benefit to AAI and may increase its utilization in patient
care.
Conclusion

These findings indicate that, while there is presumed microbial sharing between pediatric patients and therapy dogs, and while the therapy dog has the potential to serve as an intermediary vector of microbial spread, other potential transmission pathways (patient-to-patient, and environment-to-patient) also appear to contribute to microbial sharing during group AAI visits. Our results also suggest that the therapy dog could be a source of more unique microbes to patients. As hospital exposure and certain therapies decrease microbial diversity in patients, therapy dog exposure may provide a novel way to mitigate this imbalance and transmit potentially beneficial microorganisms that could be protective against hospital pathogen colonization and infection. This study shows that microbial community alterations in patients and therapy dogs during these therapy programs warrants additional research, which will make these programs safer and more sustainable.
References
Azad, M. B., Konya, T., Maughan, H., Guttmann, D. S., Sears, M. R., … Kozyrskyj, A. L. (2013). Infant gut microbiota and the hygiene hypothesis of allergic disease: Impact of household pets and siblings on microbiota composition and diversity. Allergy, Asthma and Clinical Immunology, 9(1), 1–9. https://doi.org/10.1186/1710-1492-9-15
Bert, F., Gualano, M. R., Camussi, E., Pieve, G., Voglino, G., & Siliquini, R. (2016). Animal assisted intervention: A systematic review of benefits and risks. European Journal of Integrative Medicine, 8(5), 695–706. https://doi.org/10.1016/j.eujim.2016.05.005
Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Caporaso, J. G. (2019, July). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology. https://doi.org/10.1038/s41587-019-0209-9
Boyle, S. F., Corrigan, V. K., Buechner-Maxwell, V., & Pierce, B. J. (2019). Evaluation of Risk of Zoonotic Pathogen Transmission in a University-Based Animal Assisted Intervention (AAI) Program. Front Vet Sci, 6(167). https://doi.org/10.3389/fvets.2019.00167
Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High Resolution Sample Inference from Illumina Amplicon Data. Nat Methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869.DADA2
Charry-Sanchez, J. D., Pradilla, I., & Talero-Gutierrez, C. (2018a). Animal-assisted therapy in adults: A systematic review. Complementary Therapies in Clinical Practice, 32, 169–180. https://doi.org/10.1016/j.ctcp.2018.06.011
Charry-Sanchez, J. D., Pradilla, I., & Talero-Gutierrez, C. (2018b). Effectiveness of Animal-Assisted Therapy in the Pediatric Population: Systematic Review and Meta-Analysis of Controlled Studies. Journal of Developmental and Behavioral Pediatrics, JDBP, 39(7), 580–590. https://doi.org/10.1097/DBP.0000000000000594
Dalton, K. R., Waite, K. B., Ruble, K., Carroll, K. C., DeLone, A., Frankenfield, P., … Davis, M. F. (2020). Risks Associated with Animal-Assisted Intervention Programs: A Literature Review. Complementary Therapies in Clinical Practice, 39, 101–145. https://doi.org/10.1101/2020.02.19.20025130
Davis, E. M. (2016). Gene sequence analyses of the healthy oral microbiome in humans and companion animals: A comparative review. Journal of Veterinary Dentistry, 33(2), 97–107. https://doi.org/10.1177/0898756416657239
Davis, M. F., Baron, P., Price, L. B., Williams, D. L., Jeyaseelan, S., Hambleton, I. R., … McCormack, M. C. (2012). Dry collection and culture methods for recovery of methicillin-susceptible and methicillin-resistant Staphylococcus aureus strains from indoor home environments. Applied and Environmental Microbiology, 78(7), 2474–2476. https://doi.org/10.1128/AEM.06886-11
Davis, M. F., Hu, B., Carroll, K. C., Bilker, W. B., Tolomeo, P., Cluzet, V. C., … Nachamkin, I. (2016). Comparison of culture-based methods for identification of colonization with methicillin-resistant and methicillin-susceptible staphylococcus aureus in the context of cocolonization. Journal of Clinical Microbiology, 54(7), 1907–1911. https://doi.org/10.1128/JCM.00132-16
Davis, N. M., Proctor, Di. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome, 6(1), 1–14. https://doi.org/10.1186/s40168-018-0605-2
Fadrosh, D. W., Bing Ma, P. G., Sengamalay, N., Ott, S., Brotman, R. M., & Ravel, J. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome, 2(6), 1–7.
Fall, T., Lundholm, C., Örtqvist, A. K., Fall, K., Fang, F., Hedhammar, Å., ... Almqvist, C. (2015). Early exposure to dogs and farm animals and the risk of childhood asthma. *JAMA Pediatrics, 169*(11), e153219. https://doi.org/10.1001/jamapediatrics.2015.3219

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews Microbiology, 9*(4), 244.

Havstad, S., Wegienka, G., Zoratti, E. M., Lynch, S. V., Boushey, H. A., Nicholas, C., ... Johnson, C. C. (2011). Effect of prenatal indoor pet exposure on the trajectory of total IgE levels in early childhood. *Journal of Allergy and Clinical Immunology, 128*(4), 880-885.e4. https://doi.org/10.1016/j.jaci.2011.06.039

Hoffmann, A. R., Patterson, A. P., Diesel, A., Lawhon, S. D., Ly, H. J., Stephenson, C. E., ... Suchodolski, J. S. (2014). The skin microbiome in healthy and allergic dogs. *PLoS ONE, 9*(1). https://doi.org/10.1371/journal.pone.003197

Kamioka, H., Okada, S., Tsutani, K., Park, H., Okuizumi, H., Handa, S., ... Mutoh, Y. (2014). Effectiveness of animal-assisted therapy: A systematic review of randomized controlled trials. *Complementary Therapies in Medicine, 22*(2), 371–390. https://doi.org/10.1016/j.ctim.2013.12.016

Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research, 30*(14), 3059–3066. https://doi.org/10.1093/nar/gkf436

Lefebvre, S. L., Reid-Smith, R. J., Waltner-Toews, D., & Weese, J. S. (2009). Incidence of acquisition of methicillin-resistant *Staphylococcus aureus*, *Clostridium difficile*, and other healthcare-associated pathogens by dogs that participate in animal-assisted interventions. *JAVMA, 234*(11).

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology, 15*(12), 1–21. https://doi.org/10.1186/s13059-014-0550-8

Mandhane, P. J., Sears, M. R., Poulton, R., Greene, J. M., Lou, W. Y. W., Taylor, D. R., & Hancock, R. J. (2009). Cats and dogs and the risk of atopy in childhood and adulthood. *The Journal of Allergy and Clinical Immunology, 124*(4), 745-50.e4. https://doi.org/10.1016/j.jaci.2009.06.038

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., Desantis, T. Z., Probst, A., ... Hugenholtz, P. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal, 6*(3), 610–618. https://doi.org/10.1038/ismej.2011.139

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One, 8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Misić, A. M., Davis, M. F., Tyldsley, A. S., Hodkinson, B. P., Tolomeo, P., Hu, B., ... Grice, E. A. (2015). The shared microbiota of humans and companion animals as evaluated from *Staphylococcus* carriage sites. *Microbiome, 3*(1), 1–19. https://doi.org/10.1186/s40168-014-0052-7

Morris, D. O., Lautenbach, E., Zaoutis, T., Leckerman, K., Edelstein, P. H., & Rankin, S. C. (2012). Potential for Pet Animals to Harbour Methicillin-Resistant *Staphylococcus aureus* When Residing with Human MRSA Patients. *Zoonoses and Public Health, 59*(4), 286–293. https://doi.org/10.1111/j.1863-2378.2011.01448.x

Naik, S., Bouladoux, N., Wilhelm, C., Molloy, M. J., Salcedo, R., Kastenmuller, W., ... Conlan, S. (2012). Compartmentalized control of skin immunity by resident commensals. *Science, 337*(6098), 1115–1119.
Oh, C., Lee, K., Cheong, Y., Lee, S. W., Park, S. Y., Song, C. S., ... Lee, J. B. (2015). Comparison of the oral microbiomes of canines and their owners using next-generation sequencing. PLoS ONE, 10(7), 1–15. https://doi.org/10.1371/journal.pone.0131468

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS ONE, 5(3). https://doi.org/10.1371/journal.pone.0009490

R Development Core Team. (2010). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rodrigues, A. C., Belas, A., Marques, C., Cruz, L., Gama, L. T., & Pomba, C. (2018). Risk Factors for Nasal Colonization by Methicillin-Resistant Staphylococci in Healthy Humans in Professional Daily Contact with Companion Animals in Portugal. Microbial Drug Resistance (Larchmont, N.Y.), 24(4), 434–446. https://doi.org/10.1089/mdr.2017.0063

Ross, A. A., Müller, K. M., Scott Weese, J., & Neufeld, J. D. (2018). Comprehensive skin microbiome analysis reveals the uniqueness of human skin and evidence for phyllosymbiosis within the class Mammalia. Proceedings of the National Academy of Sciences of the United States of America, 115(25), E5786–E5795. https://doi.org/10.1073/pnas.1801302115

Song, S. J., Lauber, C., Costello, E. K., Lozupone, C. A., Humphrey, G., Berg-Lyons, D., ... Knight, R. (2013). Cohabiting family members share microbiota with one another and with their dogs. eLife, (2:e00458). https://doi.org/10.7554/eLife.00458

Stein, M. M., Hrusch, C. L., Gozdz, J., Igartua, C., Pivniouk, V., Murray, S. E., ... Sperling, A. I. (2016). Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children. The New England Journal of Medicine, 375(5), 411–421. https://doi.org/10.1056/NEJMoa1508749

Swanson, K. S., Dowd, S. E., Suchodolski, J. S., Middelbos, I. S., Vester, B. M., Barry, K. A., ... Fahey, G. C. (2011). Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. ISME Journal, 5(4), 639–649. https://doi.org/10.1038/ismej.2010.162

Tun, H. M., Konya, T., Takaro, T. K., Brook, J. R., Chari, R., Field, C. J., ... Kozyrskyj, A. L. (2017). Exposure to household furry pets influences the gut microbiota of infant at 3-4 months following various birth scenarios. Microbiome, 5(1), 40. https://doi.org/10.1186/s40168-017-0254-x

Waite, T. C., Hamilton, L., & Brien, W. O. (2018). A meta-analysis of Animal Assisted Interventions targeting pain, anxiety and distress in medical settings. Complementary Therapies in Clinical Practice, 33(January), 49–55. https://doi.org/10.1016/j.ctcp.2018.07.006

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology, 73(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Ludwig, S., Jimenez-Bush, I., Brigham, E., Bose, S., Diety, G., McCormack, M. C., ... Davis, M. F. (2017). Analysis of home dust for Staphylococcus aureus and staphylococcal enterotoxin genes using quantitative PCR. Science of The Total Environment, 581, 750–755. https://doi.org/10.1016/j.scitotenv.2017.01.003

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One, 8(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Willis, A. (2019). Rarefaction, alpha diversity, and statistics. Frontiers in Microbiology, 10(2407), 1–8.
Tables and Figures

Figure 1: Microbial Pathways during Animal-Assisted Intervention Programs
Table 1: Study Population and Samples

| Study Population | All Visits | Control Visits | Intervention Visits |
|------------------|------------|----------------|--------------------|
| **Patients**     |            |                |                    |
| N total sampled  | 49 *45     | 26 (53%) ^23   | 23 (47%) ^22       |
| Male (%)         | 31 (63%)   | 15 (58%)       | 16 (69%)           |
| Age (y), mean (range) | 11.68 (1.9-20.4) | 11.07 (1.9-18.4) | 12.41 (3.5-20.4) |
| High Contact (%) | 25 (51%)   | 12 (46%)       | 13 (56%)           |
| **Visits**       |            |                |                    |
| Total            | 13         | 8 (62%)        | 5 (38%)            |
| Patients per visit, mean (range) | 3.77 (2-6) | 3.25 (2-5)    | 4.6 (3-6)         |
| **Therapy Dogs**|            |                |                    |
| N Unique Dogs    | 4          |                |                    |
| Male (%)         | 1 (25%)    |                |                    |
| Age (y), mean (range) | 6.43 (1.5-12) | | |
| **Samples**      |            |                |                    |
| From Patients    | 79         | 43 (54%)       | 36 (46%)           |
| From Dogs        | 26         | 16 (62%)       | 10 (38%)           |
| From Environment | 24         | 14 (58%)       | 10 (42%)           |
| **Total Samples**| 129        | 73 (57%)       | 56 (43%)           |
| Field Blanks     | 12         | 7 (58%)        | 5 (42%)            |
| Laboratory Controls | 21     |                |                    |
| **Total Controls**| 33         |                |                    |

*45 patients with microbial samples collected, 23 in control and 22 in intervention
**Figure 2: Relative Abundance of Top 20 Genera**

### A. Relative Abundance by Sample Host

**Genus**
- Other
- Veillonella
- Streptococcus
- Staphylococcus
- Moraxella
- Lactobacillus
- Coriobacterium
- Anaerococcus
- Akkermansia

### B. Patients in Control Visits

### C. Patients in Intervention Visits

*** Benjamini-Hochberg adjusted p-values <0.001 for differential abundant genera using a negative binomial model (DESeq) between sample sites

**Within Patients:** Blue *** = higher in post samples, Red *** = higher in pre samples

Mean total DNA concentration in patients in control = 6.28, in intervention = 4.42 (ng/ul)

Mean qPCR 16S gene copies in patients in control = 22254, in intervention = 8691 (/ul DNA)
Figure 3: Alpha Diversity by Sample Host and Site, and Within Patient Samples

In Patients:

A. Total Taxa

B. Shannon Diversity

C. Faith’s Phylogenetic Diversity

In Dogs and Environment:

D. Total Taxa

E. Shannon Diversity

F. Faith’s Phylogenetic Diversity

Thin lines = within subject changes, bold lines = aggregated group means
** Kruskal-Wallis test p<0.05 for median difference in change in alpha diversity level (post-pre) in control vs intervention (in high contact patients and dogs)
Figure 4: Beta Distance for Microbial Composition Difference, by Contact Level and Visit Type (post – pre visit)

PERMANOVA model p value results for difference in microbial composition beta distance between patients pre compared microbial composition beta distance between patients post visit (kid-kid) or difference in microbial composition beta distance between patients and therapy dogs pre compared microbial composition beta distance between patients and therapy dogs post visit (kid-dog), within each stratification (visit type and contact level).

Refer to Supplement Figure 3&4 for example calculations and pre/post distances

**BOLD** FDR-corrected p <0.005