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TITLE: Composition, Function, and Role of the Intestinal Microbiome in Pediatric Heart Failure and Heart Transplantation

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CONTRACTING ORGANIZATION: Baylor College of Medicine

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14. ABSTRACT The role of the intestinal microbiome has gained substantial interest as a novel marker for prognosis of disease and as a potential target for therapeutic intervention. There is evidence that the microbiome exerts a fundamental influence on immunity, can be altered in heart failure, and can be further disrupted by immunosuppressive medications. This indicates the potential significant impact that alterations to the intestinal microbiome may play in the care of children who have undergone heart transplant. The composition, function, and role of the intestinal microbiome in children with congenital heart disease or heart transplant is not currently known. The main objective of this research is to characterize and investigate the role of the intestinal microbiome in this population and generate the preliminary data necessary to determine effect estimates that will be used to power large prospective randomized studies of targeted microbial restoration. Patient recruitment and stool collection is still ongoing. Preliminary analyses indicate that there are significant microbial compositional shifts after patients undergo heart transplant. Furthermore, heart transplant patients with post-transplant complications have a significantly different microbial composition compared to those without complications. As this study is prospective in nature, there is still on-going follow up and stool collection and analysis to determine associations between specific microbial changes and specific post-transplant complications.						
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1. INTRODUCTION:

The role of the intestinal microbiome has gained substantial interest as a novel marker for diagnosis and prognosis of disease and as a potential target for therapeutic intervention. There is evidence that the microbiome exerts a fundamental influence on innate and adaptive immunity, can be altered in heart failure, can shift rapidly during intestinal ischemia and reperfusion, and can be further disrupted by immunosuppressive medications. This indicates the potential significant impact that alterations to the intestinal microbiome may play in the care of children who have undergone heart transplant. Furthermore, the success of fecal microbial transplant in patients with *Clostridium difficile* diarrhea has demonstrated that the microbiome is potentially modifiable and indicates the therapeutic potential of microbiome restoration to improve the duration and severity of diarrheal disease. The composition, function, and role of the intestinal microbiome in children and young adults with congenital heart disease or heart transplant is not currently known. Our **long-term goal** is to identify modifiable risk factors and develop innovative treatment strategies to improve outcomes for these patients. The **main objective** of this research is to characterize and investigate the role of the intestinal microbiome in this population and generate the preliminary data necessary to determine effect estimates that will be used to power large prospective randomized studies of targeted microbial restoration. Characterizing the intestinal microbiome in this patient population offers significant potential to greatly impact and improve the health outcomes of individuals with congenital heart disease. Improving post-heart transplant outcomes can also ameliorate the supply-and-demand mismatch crisis of donor organ allocation by reducing the need for re-transplantation.

KEYWORDS:

Intestinal Microbiome, Metabolomics, Pediatric Cardiology, Congenital Heart Disease, Heart Transplant, Diarrhea

2. ACCOMPLISHMENTS:

What were the major goals of the project?

The three major goals of this project are to:

- 1) Prospectively document and compare alterations in the composition and diversity of the intestinal microbiome and associated metabolome in children and young adults listed for heart transplant to the intestinal microbiome and associated metabolome in healthy, age- and sex-matched controls.
- 2) Prospectively document and compare alterations in the composition and diversity of the intestinal microbiome and associated metabolome in children and young adults before and after placement of a ventricular assist device or heart transplant.
- 3) Evaluate the association of alterations in the composition and diversity of the intestinal microbiome and associated metabolome in children and young adults with the following post-heart transplant outcomes: diarrhea, systemic infection, coronary allograft vasculopathy, graft rejection, graft failure, and re-transplant or death.

What was accomplished under these goals?

The main objective of this proposal is to characterize and investigate the role of the intestinal microbiome in children that undergo heart transplantation and generate the preliminary data necessary to determine effect estimates that will be used to power large prospective randomized studies of targeted microbial restoration. The major activities that have occurred during this reporting period have included additional identification, recruitment, and enrollment of eligible participants and additional stool collection. The target enrollment by the end of Year 2 was projected to be 145 patients. To date, 154 patients have been enrolled in the study, which exceeds our goal. Each patient has filled out a pain/stool diary and nutritional recall survey, and each of these surveys have been entered into our database. From these patients, 316 stool samples have been collected from 105 patients. This includes samples from 30 patients with pre-transplant samples who have undergone transplant and from whom sequential post-transplant samples have been obtained. Twelve of the 150 patients have been supported with a ventricular assist device.

Patient characteristics that are recorded include age, gender, race/ethnicity, type of congenital heart disease or cardiomyopathy, and medications and dosage at time of stool collection. In addition, prospective clinical outcomes are being collected and entered into our database. Multiple patient outcomes including diarrhea, infection, coronary allograft vasculopathy, graft rejection, graft failure, and death have occurred.

To date, 150 samples have undergone DNA extraction, processing, and sequencing. As stated in the approved SOW, the goal was to have 120 pre-heart transplant samples, 40 post-ventricular assist device samples, and 195 post-heart transplant samples. As stated in the approved SOW, data analysis and dissemination was to occur during Year 2.

At the end of Year 1, we had collected a total of 130 stool samples. During the last reporting period, we requested and received a no-cost extension in order to permit more time to collect additional stool samples. It was initially anticipated that we would collect close to 500 stool samples by the end of the study period.

In response to the COVID-19 Pandemic, Baylor College of Medicine instituted college wide measures to help limit the spread of the virus and perform responsible conduct of research. Starting in March 2020, the College limited access to research facilities and implemented a plan with phased increases of access as recovery efforts commenced. Following the OMB Flexibility guidelines, researchers were retained on grants during this period when they had both continuity support and direct activities in support of the grant.

As per the approved SOW, the plan was to perform data analysis during months 15-18. This allows for quality control to analyze the stool samples in large batches. However, due to the concerted effort at Baylor College of Medicine and Texas Children's Hospital to follow social distancing recommendations, limited access to campus facilities (including research facilities), postponement of patients' clinic appointments, postponement of routine cardiac catheterizations, and a transition to "virtual" patient encounters, the number of new stool samples and further bacterial DNA extraction, processing, sequencing, and analysis has been significantly affected.

There continues to be limited access to campus facilities, but the aims continue to be studied as originally proposed and patients' clinic appointments and cardiac catheterization procedures are increasingly being performed closer to pre-COVID-19 levels. Stool collection is still ongoing

although no additional patients have been recruited or enrolled since March 2020. At 24 months, we have collected 316 stool samples, including 186 samples since the last reporting period.

Since final data analysis has not yet been performed, there are no conclusions to provide. However, some preliminary results are included below. To date, 150 samples have undergone bacterial DNA extraction. The resulting nucleic acid was processed through an Illumina MiSeq 16S sequencing pipeline. Two separate regions of the highly variable areas of the 16S rRNA gene, V1V3 and V4, were targeted for sequencing. Resulting raw sequences were analyzed via the standard analysis pipeline, which utilizes the UPARSE algorithm for clustering of sequences into operational taxonomic units (OTUs) and the SILVA database for taxonomic classification of each OTU. Metabolomic analyses was performed using p180 Kits (Biocrates) on the Ultra-Performance Liquid Chromatography tandem mass spectrometer (AbSciex 6500).

Pre-transplant (Pre-HTx) patients have a different composition compared to post-transplant (Post-HTx) patients at both the phyla and genus levels (Figure 1A and Figure 1B), and patients with adverse clinical “events” have a different composition compared to asymptomatic or “routine” patients at both the phyla and genus levels (Figure 2A and 2B). There are also multiple specific microbial genus level compositional differences (Figures 3A and 3B). As this study is prospective in nature, we will continue to follow these patients to determine associations between specific compositional changes and clinical events/adverse outcomes.

The number of collected stool specimens since March 2020 has significantly declined, so it is possible we may not be able to obtain the goal of ~500 stool samples by the completion of the study, including the 1-year no cost extension.

Figure 1A

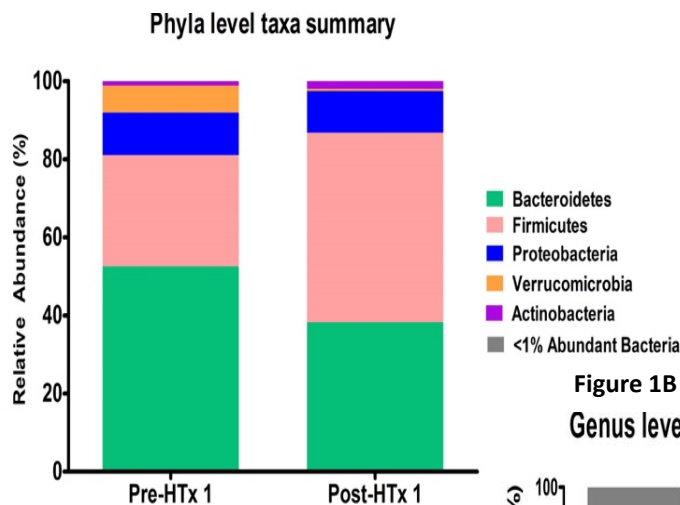


Figure 1B

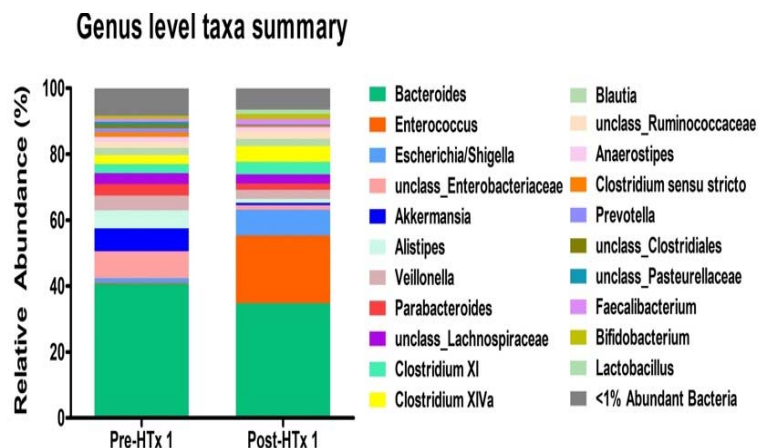


Figure 2A

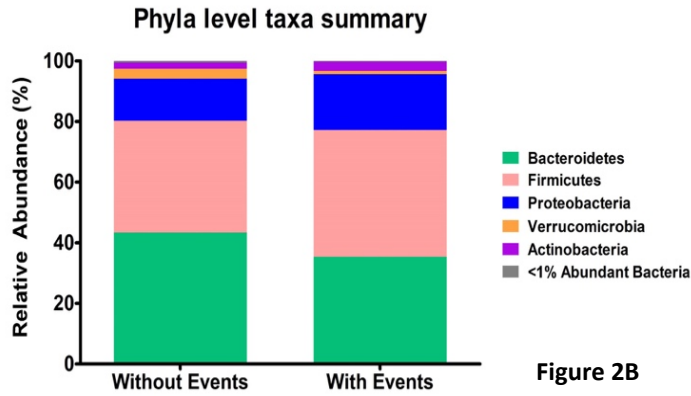


Figure 2B

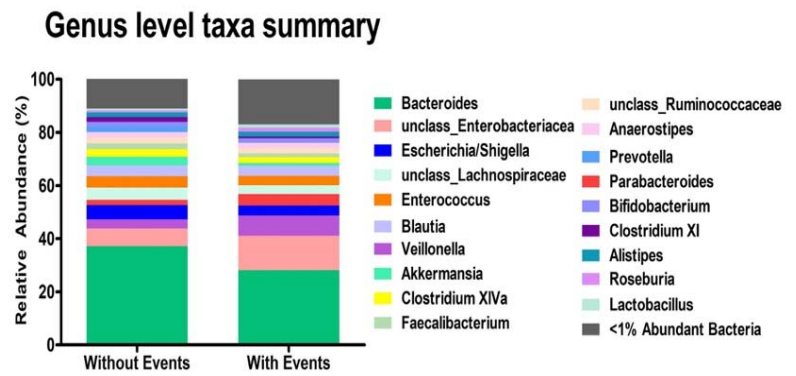


Figure 3A

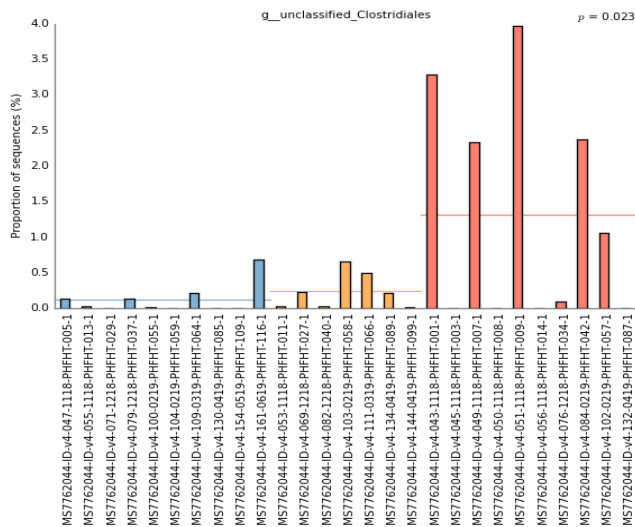
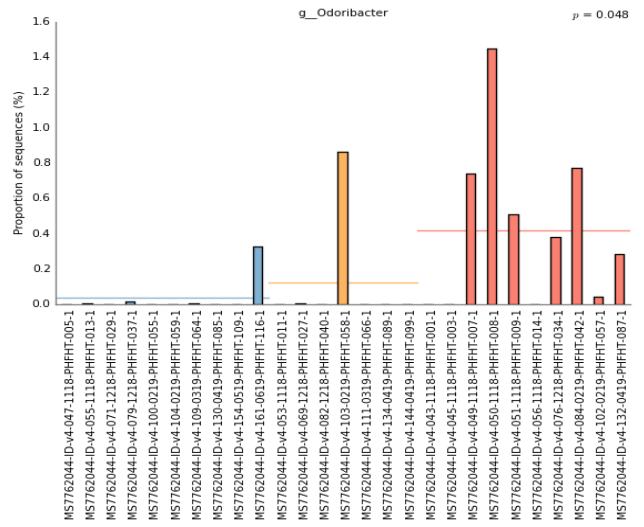


Figure 3B



What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

An abstract entitled “Intestinal Microbiome Composition Changes After Heart Transplant” was accepted to the 2020 International Society for Heart and Lung Transplantation Annual Meeting and Scientific Sessions in Montreal. Due to COVID-19 the meeting was cancelled.

What do you plan to do during the next reporting period to accomplish the goals?

We will continue to prospectively collect stool samples from the enrolled patients. We anticipate we will soon be able to collect more samples as patients’ clinic appointments and cardiac catheterization procedures are increasingly being performed closer to pre-COVID-19 levels.

In addition, we anticipate that we will soon have pre-COVID-19 level access to research facilities as Baylor College of Medicine and Texas Children’s Hospital continue with phased increases of access. This will permit us to perform DNA extraction, sequencing, and analysis on the over 100 stool samples currently frozen.

During the next reporting period, we plan to perform data analysis so we may provide results and conclusions at the next report. We also plan to submit an additional no cost extension request if we are unable to collect the projected number of stool samples and/or have insufficient access to our research facility resources due to the circumstances of the pandemic.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

During the last reporting period, we amended the protocol to include patients under 1 year of age. Children under 1 year of age made up the largest age demographic of patients awaiting pediatric heart transplantation. The reason for this was to increase the number of enrolled patients and stool samples to collect and analyze.

Actual or anticipated problems or delays and actions or plans to resolve them

As mentioned previously, in response to the COVID-19 Pandemic, Baylor College of Medicine instituted college wide measures to help limit the spread of the virus and perform responsible conduct of research. Beginning in March 2020, limited access to research facilities was implemented with phased increases of access. Due to the concerted effort at Baylor College of Medicine and Texas Children's Hospital to follow social distancing recommendations, limited access to campus facilities (including research facilities), postponement of patients' clinic appointments, postponement of routine cardiac catheterizations, and a transition to "virtual" patient encounters, the number of new stool samples and further bacterial DNA extraction, processing, sequencing, and analysis has been affected. This has led to a delay in further processing and analysis of samples.

During the last reporting period, we anticipated we would not be able to collect and then analyze as many stool samples as we initially projected. We therefore requested and received a 1-year no cost extension to allow more time to collect and then analyze stool samples.

There continues to be limited access to campus facilities, but the aims continue to be studied as originally proposed and patients' clinic appointments and cardiac catheterization procedures are increasingly being performed closer to pre-COVID-19 levels. Stool collection is still ongoing, and we plan to process and analyze the stool specimens during the next reporting period.

We also plan to submit an additional no cost extension request if we are unable to collect the projected number of stool samples and/or have sufficient access to our research facility resources due to the circumstances of the pandemic.

Changes that had a significant impact on expenditures

We were able to find and utilize sample collection kits from a different vendor at a reduced cost, saving us approximately \$9300. This will free up additional funds to allow for more detailed and more sophisticated microbiome and metabolomic analysis (approximately \$7500). We also plan to disseminate our results at multiple medical meetings and plan to allocate the remaining additional funds for conference travel and publication costs.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Significant changes in use or care of human subjects

As stated above, we amended the protocol to include patients under 1 year of age. Otherwise, there have been no significant changes.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS:

Publications, conference papers, and presentations

An abstract entitled “Intestinal Microbiome Composition Changes After Heart Transplant” was accepted to the 2020 International Society for Heart and Lung Transplantation Annual Meeting and Scientific Sessions in Montreal. Due to the COVID-19 pandemic, the meeting was cancelled.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Joseph Spinner, MD

Project Role: PI

Researcher Identifier (e.g. ORCID ID): <https://orcid.org/0000-0001-9539-6252>

Nearest person month worked: 3

Contribution to Project: Dr. Spinner is the project lead & is responsible for the design, implementation, & deliverables.

Funding Support: Baylor College of Medicine Pediatric Cardiology covers salary & protected time for project

Name: Sridevi Devaraj

Project Role: Co-Investigator

Researcher Identifier (e.g. ORCID ID): <https://orcid.org/0000-0001-9189-7914>

Nearest person month worked: < 1

Contribution to Project: Dr. Devaraj is a co-investigator responsible for sample extractions, sequencing, & metabolomics testing. She also interprets the statistical analysis performed by the biostatisticians at the TCH Microbiome Center.

Funding Support: None

Name: Ayesha Masood

Project Role: Research Coordinator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 3

Contribution to Project: Ms. Masood assists with patient identification, screening, & enrollment. She consents patients, collects records, diaries, samples & surveys, & keeps the database up to date.

Funding Support: None

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable.

QUAD CHARTS: Not applicable.

9. APPENDICES: None.