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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, Houston, TX

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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.

15. SUBJECT TERMS

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

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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 DNA extraction, processing, sequencing, and preliminary analyses of previously frozen stool samples and clinical follow up of the patients in whom stool samples have been collected. The target enrollment for the study 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, 300 stool 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. Due to issues encountered during the COVID-19 pandemic described below, we were not able to obtain the goal number of samples.

During the last reporting period, we requested and received a no-cost extension in order to permit more time to process and analyze stool samples and continue to perform data analysis with biostatistical support.

In response to the continuation of the COVID-19 Pandemic, Baylor College of Medicine continued 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. Research staff continued to have limited access to perform in-person patient enrollment, survey collection, and stool collection due to Texas Children's Hospital restrictions and social distancing mitigation policies.

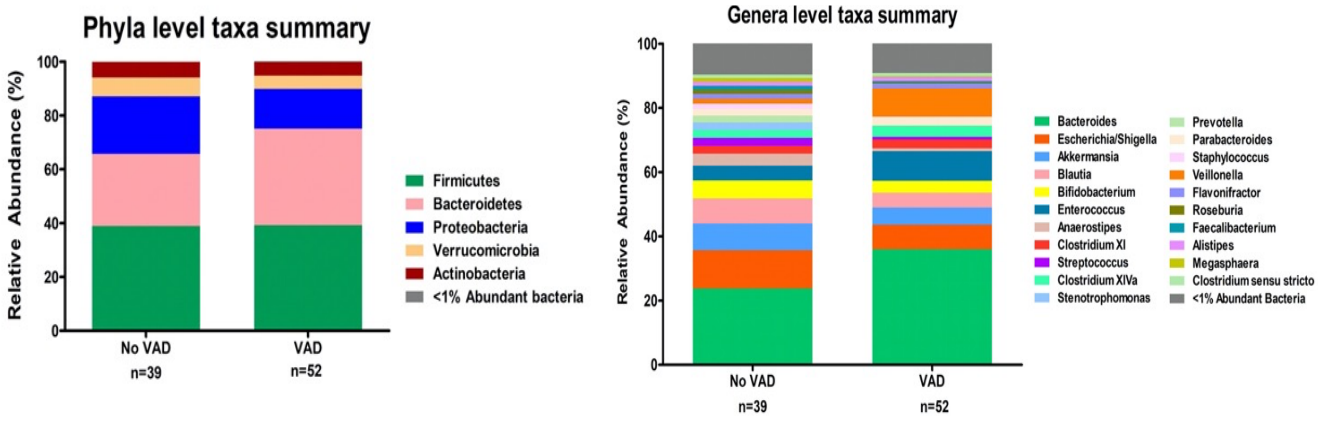
As per the approved SOW, the plan was to perform data analysis during months 15-18. Performing microbial DNA extraction and sequencing in large batches at the same time allows for quality control to most optimally analyze the stool samples. However, due to the concerted effort at Baylor College of Medicine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

Despite the restrictions encountered during the COVID-19 Pandemic, we still collected a total of 316 stool samples. Since the last reporting period, 150 stool samples have undergone DNA extraction and sequencing. During the study period, the enrolled patients have been followed clinically. Over 75 clinically significant "events" have occurred during the follow-up time.

Since final data analysis is still ongoing, there are no conclusions to provide. However, some preliminary results are included below. To date, 300 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).

When assessing samples obtained from pre-transplant (Pre-HTx) patients, we have identified compositional differences at both the phyla and genus levels between patients supported on a ventricular assist device (VAD) vs those patients not supported on a VAD (Figure 1A and Figure 1B). Notable differences included the differences in the abundance of *Bacteroides, Veillonella*, and *Anaerostipes* species.

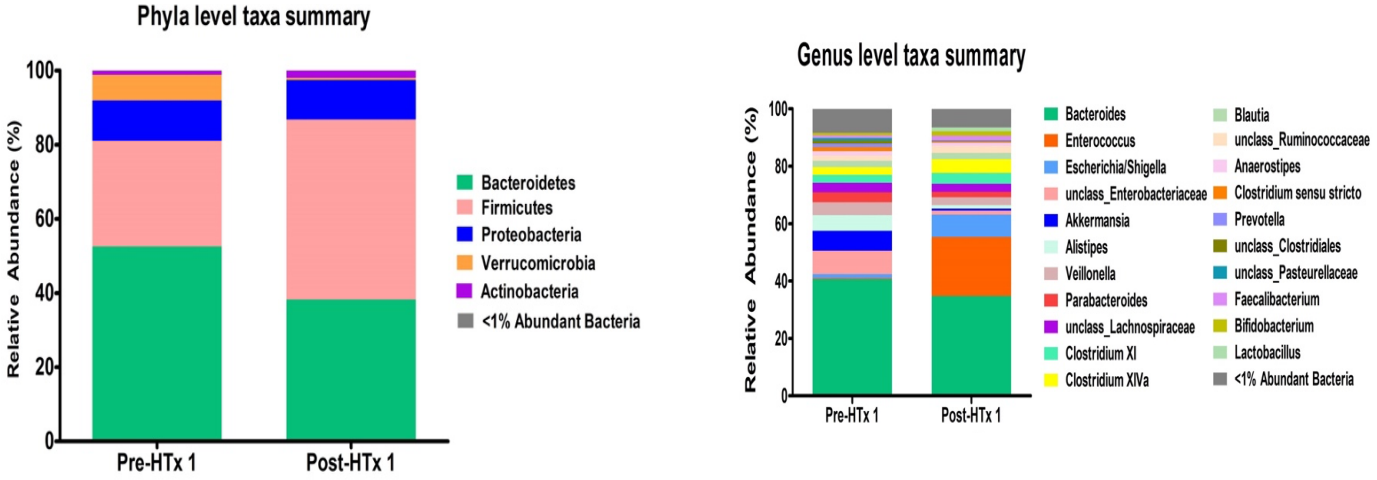
Figures 1A and 1B: Compositional differences among patients waiting for heart transplant: VAD vs no VAD



VAD: ventricular assist device

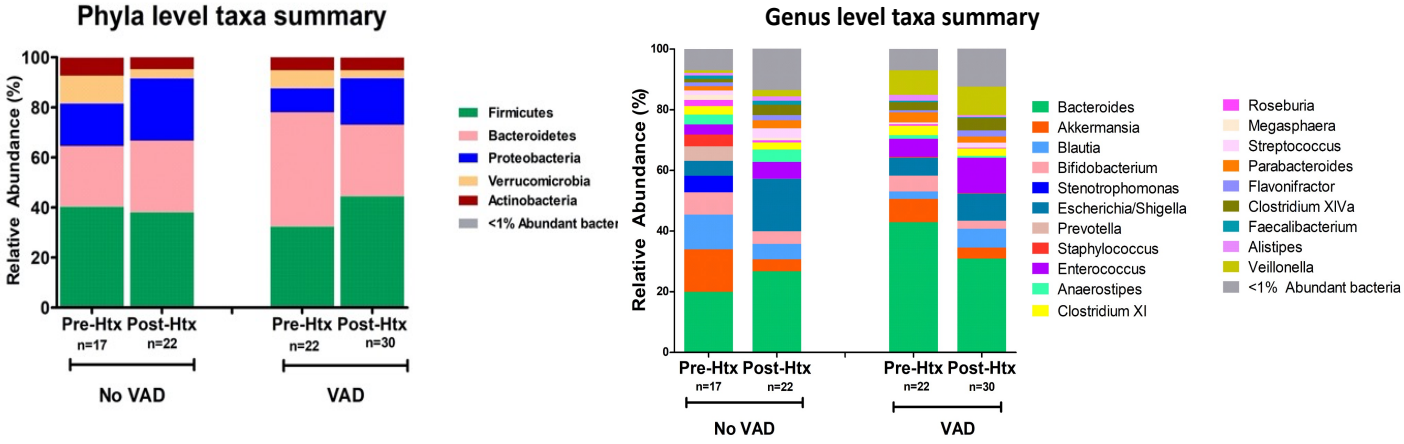
When comparing the composition of the intestinal microbiome between Pre-HTx and Post-HTx patients, we identified compositional differences at both the phyla and genus levels (Figure 2A and 2B). Notable differences included the differences in the abundance of *Bacteroides, Enterococcus*, and *Akkermansia* species. Considering the previous findings of compositional differences existing between Pre-HTx patients on a VAD vs those that were not, we also compared post-HTx samples specifically between those with prior VAD support vs those without prior VAD support. Again, we found compositional differences at the phyla and genus levels (Figures 3A and 3B).

Figures 2A and 2B: Compositional differences between Pre-HTx vs Post-HTx patients



Pre-HTx: pre-transplant Post-HTx: post-transplant

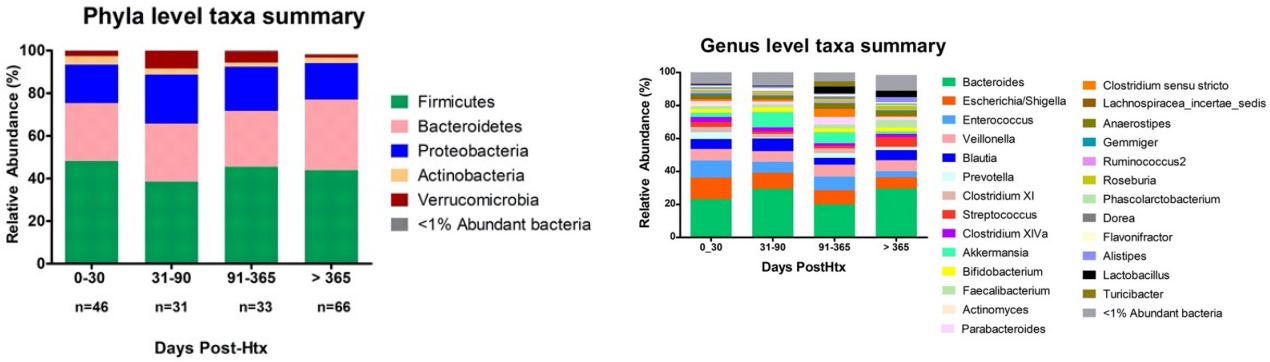
Figures 3A and 3B: Compositional differences among Pre-HTx vs Post-HTx patients based on VAD support



Pre-HTx: pre-transplant Post-HTx: post-transplant VAD: ventricular assist device

As part of the study, the patients were prospectively enrolled pre-transplant and serial stool samples were collected post-transplant. There appear to be compositional shifts depending on time elapsed from transplant (Figures 4A and 4B). Further analysis is ongoing.

Figures 4A and 4B: Serial microbial compositional changes post-transplant



Post-HTx: post-transplant

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

Nothing to report.

How were the results disseminated to communities of interest?

We previously presented an abstract entitled "Intestinal Microbiome Composition Changes After Heart Transplant" at the 2020 International Society for Heart and Lung Transplantation Annual Meeting and Scientific Sessions. We are in the process of submitting abstracts for the next International Society for Heart and Lung Transplantation Annual Meeting and Scientific Sessions (submission deadline is in October 2021).

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

During the next reporting period, we plan to complete our data analysis so we can provide conclusions at the next report and disseminate our results through published manuscripts.

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

Nothing to report since the last reporting period.

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, there was limited access to campus facilities (including research facilities) for research staff, and there was a transition to more "virtual" patient encounters. This resulted in fewer stool samples being obtained than was the initial goal as specified in the SOW. Performing DNA extraction, sequencing, and analysis was also delayed and limited during this time. We therefore requested and received a no cost extension and previously expanded our patient population to include children under 1 year of age.

The aims continue to be studied as originally proposed, and we are in the process of completing our analyses now.

Changes that had a significant impact on expenditures

Nothing to report since last reporting period.

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

During a prior reporting period, we amended the protocol to include patients under 1 year of age. There were no changes during this reporting period.

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. We are in the process of submitting abstracts for presentations at the

next International Society for Heart and Lung Transplantation Annual Meeting and Scientific Sessions (submission deadline is in October 2021). Manuscripts are also in process.

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: 2
Contribution to Project: Ms. Masood assisted with data collection and helped keep the database up to date.
Funding Support: None

Name: Kelli Noon
Project Role: Research Nurse
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: Ms. Noon assisted with clinical data collection and helped keep the database up to date after the departure of Ms. Masood
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?

Dr. Spinner (PI) is now a local site-PI on a multi-institutional study detailed below. His effort is 0.48 calendar months.

62609691-208481 (Rosenthal) 01/01/2021 – 12/31/2022 0.48 CM
Enduring Hearts/Additional Ventures Total costs
Can HLA data be used to support Precision Medicine in Pediatric Heart Transplantation? An exploratory analysis.
Role: Site PI

Dr. Spinner is now a collaborating investigator on a study detailed below. His effort is 0.21 calendar months.

(Martin) 07/01/2021 – 06/30/2024 0.21 CM
American Heart Association/Enduring Hearts Total costs
Early Detection of Cardiac Allograft Vasculopathy in post-transplant pediatric hearts via single-cell RNA Sequencing
Role: Collaborating Investigator

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.