Impact of enteric parasites on intestinal microbiota diversity and metagenomic changes in Argentinian children

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Abstract

Next generation sequencing (NGS) for microbiome analysis is commonly performed using 16S rRNA gene sequencing or whole genome shotgun sequencing (WGS). We carried out both WGS and 16S sequencing on human fecal samples from a 1-2 age group cohort focusing on two groups: helminth infected (Ascaris, Ancylostoma, Necator, Strongyloides, and Trichuris) versus non-infected (no-parasite) individuals verified by multi-parallel real-time quantitative PCR. WGS approach provided higher resolution allowing classification to the bacterial strain level and in some cases even sub-strain level. 16S sequencing could not provide resolution below genus level. Both methods demonstrated similar sensitivity to detect Shannon alpha diversity differences. While there were no statistical differences within the helminth-infected group (p > 0.05) or no-parasite group (p > 0.05), WGS showed a significant increase in diversity in terms of different species (DNA) as compared to 16S rRNA gene sequencing. OSM provides a measure of the change in proportion of specific bacterial sequences for helminths and non-parasite groups. This measure is useful for determining the capacity of an assay to discriminate between 2 experimental groups and small effect size. The WGS method provides rich metagenomic functional information as compared to 16S rRNA sequencing. Metagenomic functional information for 16S RNA reads can be inferred using PICRUSt software through taxonomic information but lacks the direct evidence of genes found in WGS. On the other hand, 16S sequencing is computationally expensive, while WGS data are challenging to manage and require software with complex algorithms. Our results show important information for selecting the optimal assay based on function and price with implications in evolutionary investigations and tropical medicine.

Introduction

- >2 billion Gi parasite infections worldwide in poorest and resource-poorer communities
- Gi parasites may disrupt normal intestinal microbiota
- Decreased microbial biodiversity is associated with disease, including:
  - Malabsorption
  - Inflammatory bowel diseases
- Vitamin B12 involved in metabolism of every human cell
- Bacteria have the enzymes needed for vitamin B12 biosynthesis
- qPCR is rapid, quantitative, high-throughput and is a more reliable species-specific method

Materials and methods

- Field site: Orán, Argentina
- Peri-urban community
- Temperate climate
- 99 patient samples
- Asymptomatic children
- Ages 4-6 years old
- No recent antibiotics
- qPCR and microscopy for presence of:
  - Ascaris lumbricoides
  - Strongyloides stercoralis
  - Ancylostoma duodenale
  - Giardia lamblia
  - Necator americanus
  - Cryptosporidium species
  - Trichuris trichiura
  - Entamoeba histolytica
- NEBNext® Microbiome DNA Enrichment Kit
- NEBNext® Ultra™ DNA Library Prep Kit for Illumina®
- Illumina NextSeq® Whole genome sequencing
- Livermore Metagenomics Analysis Toolkit (LMAT) and Diamond software
- Phred quality score 20 (99% base call accuracy)
- Normalized to 10,000 reads for bacterial diversity

Results

- Increasing Giardia burden (fg/µl) correlates to decreasing intestinal bacterial diversity
  - Spearman r = -0.5491
  - p = 0.0244

- Giardia group had more anaerobic bacteria than other cohorts optimizing conditions for Prevotella
  - (p = 0.012)
  - Giardia infections had lower cellular amino acid metabolic processes than helminth infections
  - (p = 0.047)

- Giardia >1 fg/µl group had more abundant Prevotella than No Parasite group
  - (p = 0.037) (A) with Helminths group decreased Prevotella to Giardia group
  - (p = 0.024) and Giardia/helminth co-infected negating these differences

- High Giardia infected children had decreased cobalamin biosynthesis genes compared to No Parasites
  - (p = 0.038)(A) with compensatory effects from Helminth infections
  - (p = 0.021)(B)

Conclusions

- Higher Giardia burdens correlate to less bacterial diversity which could indicate worse disease status
- These findings are mostly with higher Giardia burdens and likely due to changes in intestinal micro-environments due to Giardia creating anaerobic microenvironments
- Giardia infected group had significant increases in Prevotella species compared to helminth groups
- Coinfections negated these differences
- Metagenomics showed lower cellular amino acid processes and decreased cobalamin (Vitamin B12) biosynthesis genes in Giardia infected children and related to high Giardia burden
- Useful for epidemiology and morbidity studies
- Correlate mechanism of decreased Vitamin B12 genes to growth delays in children infected with intestinal parasites
- Expanding understanding of morbidity and malnutrition
- Future directions:
  - Correlate quantity of parasite DNA with clinical outcomes
  - Associate morbidity to changes in microbiome
  - Treat children with anti-parasitics and evaluate changes in microbiome

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