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GUT HEALTH DIGEST

UNIVERSITY OF WASHINGTON STRATEGIC ANALYSIS, RESEARCH & TRAINING (START) CENTER

REPORT TO THE BILL & MELINDA GATES FOUNDATION

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DETAILS OF ARTICLES

1. Dynamics of metatranscription in the inflammatory bowel disease gut microbiome.
   Schirmer M, Franzosa EA, Lloyd-Price J, McIver LJ, Schwager R, Poon TW, *et al*.
   Nature Microbiology. 08 January 2018. [Epub ahead of print].
   PubMed ID: 29311644

ABSTRACT
Inflammatory bowel disease (IBD) is a group of chronic diseases of the digestive tract that affects millions of people worldwide. Genetic, environmental and microbial factors have been implicated in the onset and exacerbation of IBD. However, the mechanisms associating gut microbial dysbioses and aberrant immune responses remain largely unknown. The integrative Human Microbiome Project seeks to close these gaps by examining the dynamics of microbiome functionality in disease by profiling the gut microbiomes of >100 individuals sampled over a 1-year period. Here, we present the first results based on 78 paired faecal metagenomes and metatranscriptomes, and 222 additional metagenomes from 59 patients with Crohn's disease, 34 with ulcerative colitis and 24 non-IBD control patients. We demonstrate several cases in which measures of microbial gene expression in the inflamed gut can be informative relative to metagenomic profiles of functional potential. First, although many microbial organisms exhibited concordant DNA and RNA abundances, we also detected species-specific biases in transcriptional activity, revealing predominant transcription of pathways by individual microorganisms per host (for example, by *Faecalibacterium prausnitzii*). Thus, a loss of these organisms in disease may have more far-reaching consequences than suggested by their genomic abundances. Furthermore, we identified organisms that were metagenomically abundant but inactive or dormant in the gut with little or no expression (for example, *Dialister invisus*). Last, certain disease-specific microbial characteristics were more pronounced or only detectable at the transcript level, such as pathways that were predominantly expressed by different organisms in patients with IBD (for example, *Bacteroides vulgatus* and *Alistipes putredinis*). This provides potential insights into gut microbial pathway transcription that can vary over time, inducing phenotypical changes that are complementary to those linked to metagenomic abundances. The study's results highlight the strength of analysing both the activity and the presence of gut microorganisms to provide insight into the role of the microbiome in IBD.

DOI: 10.1038/s41564-017-0089-z
IMPACT FACTOR: *NA*
CITED HALF-LIFE: 0.5

START COMMENTARY: This article utilized shotgun metagenomics and metatranscriptomics to explore the relationship between microbial abundance (the metagenome) and functional activity (the metatranscriptome) in individuals with and without IBD (UC or Crohn's). The study provides insights into the relationship between these two measures during diseased and non-diseased states and suggests that solely measuring gut microbial diversity through metagenomics may misrepresent the importance of different microbial species. As can be seen in Figure 2a, while many individual species showed strong correlations between their metagenomics and metatranscriptomic activities, some key species were discordant. For example, *F. prausnitzii,* which produces butyrate, was relatively stable in different patients when measured by metagenomics, but this was not predictive of the metatranscriptome activity, which differed between disease states. Figure 3 highlights additional differences between species-specific metagenomics function potential and metatranscriptomic functional activity. This study suggests that metagenomics analysis is not always appropriate when looking for differences in gut microbiota and a metatranscriptome approach provides more useful data.

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2. Relationship between growth and illness, enteropathogens and dietary intakes in the first 2 years of life: findings from the MAL-ED birth cohort study.
   MAL-ED Network Investigators.
   BMJ Global Health. 2(4). 2017 December 28.
   PubMed ID: 29333282

ABSTRACT
BACKGROUND: Dietary and illness factors affect risk of growth faltering; the role of enteropathogens is less clear. As part of the Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study, we quantify the effects of enteropathogen infection, diarrhoea and diet on child growth.
METHODS: Newborns were enrolled and followed until 24 months. Length and weight were assessed monthly. Illnesses and breastfeeding practices were documented biweekly; from 9 to 24 months, non-breast milk intakes were quantified monthly. Routinely collected non-diarrhoeal stools were analysed for a broad array of enteropathogens. A linear piecewise spline model was used to quantify associations of each factor with growth velocity in seven of eight MAL-ED sites; cumulative effects on attained size at 24 months were estimated for mean, low (10th percentile) and high (90th percentile) exposure levels. Additionally, the six most prevalent enteropathogens were evaluated for their effects on growth.
RESULTS: Diarrhoea did not have a statistically significant effect on growth. Children with high enteropathogen exposure were estimated to be 1.21±0.33 cm ($p<0.001$; 0.39 length for age (LAZ)) shorter and 0.08±0.15 kg ($p=0.60$; 0.08 weight-for-age (WAZ)) lighter at 24 months, on average, than children with low exposure. *Campylobacter* and enteroaggregative *Escherichia coli* detections were associated with deficits of 0.83±0.33 and 0.85±0.31 cm in length ($p=0.011$ and 0.001) and 0.22±0.15 and 0.09±0.14 kg in weight ($p=0.14$ and 0.52), respectively. Children with low energy intakes and protein density were estimated to be 1.39±0.33 cm ($p<0.001$; 0.42 LAZ) shorter and 0.81±0.15 kg ($p<0.001$; 0.65 WAZ) lighter at 24 months than those with high intakes.
CONCLUSIONS: Reducing enteropathogen burden and improving energy and protein density of complementary foods could reduce stunting.

DOI: 10.1136/bmjgh-2017-000370
IMPACT FACTOR: 2.2
CITED HALF-LIFE: 2.9

START COMMENTARY: This is the latest analysis to come out of the MAL-ED birth cohort study. The authors used a linear piecewise spline model to look at the associations between enteropathogen exposure, dietary, and socioeconomic risk factors and growth. This modelling approach allowed investigators to look at the effect of different risk factors on period-specific growth rates that naturally vary with age in the first two years of life. The authors observed that there was a negative relationship between enteropathogen detection and linear growth rate within a given period, but then this often rebounded in the following period, although infants never reached full recovery compared to non-exposed peers. There was a small negative association observed between growth rate and diarrhea episodes not associated with antibiotic use, suggesting prompt treatment of episodes may limit the negative effects of infection resultant in diarrhea. The authors suggested that the overall limited association seen between diarrheal episodes and growth may be due to the lower diarrhea incidence from the higher level of sanitation (Table 1) and increased healthcare access in the study populations compared with historical study populations.

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3. Metatranscriptome of human faecal microbial communities in a cohort of adult men.
   Abu-Ali GS, Mehta RS, Lloyd-Price J, Mallick H, Branck T, Ivey KL.
   Nature Microbiology. 2018 January 15. [Epub ahead of print].
   PubMed ID: 29335555

ABSTRACT
The gut microbiome is intimately related to human health, but it is not yet known which functional activities are driven by specific microorganisms' ecological configurations or transcription. We report a large-scale investigation of 372 human faecal metatranscriptomes and 929 metagenomes from a subset of 308 men in the Health Professionals Follow-Up Study. We identified a metatranscriptomic 'core' universally transcribed over time and across participants, often by different microorganisms. In contrast to the housekeeping functions enriched in this core, a 'variable' metatranscriptome included specialized pathways that were differentially expressed both across participants and among microorganisms. Finally, longitudinal metagenomic profiles allowed ecological interaction network reconstruction, which remained stable over the six-month timespan, as did strain tracking within and between participants. These results provide an initial characterization of human faecal microbial ecology into core, subject-specific, microorganism-specific and temporally variable transcription, and they differentiate metagenomically versus metatranscriptomically informative aspects of the human faecal microbiome.

DOI: 10.1038/s41564-017-0084-4
IMPACT FACTOR: *NA*
CITED HALF-LIFE: 0.5

START COMMENTARY: This is one of three articles published in *Nature Microbiology* which examined the metatranscriptome of the microbiota. Authors in this study examined the metatranscriptome in fecal samples from 308 healthy men provided at up to four different time points (2 pairs of samples collected within 48 hours of each other). In initial analyses, authors found that only 44% of DNA core pathways in the microbial species were actively transcribed in this cohort. Figure 4 C and D compare the metagenomics potential and metatranscriptomic activity for two core pathways expressed in two different subjects. This figure highlights the authors' observation that different microbial species contributed to transcribing the same core pathways among subjects. The authors hypothesized that microbial decision-making on transcription is driven by a functional ecological model, and certain members of an individual's microbial community dominate transcription of these core functions at any point in time. As in the other metatranscriptome paper presented this month, this study highlights the value of metatranscriptome analysis in future gut microbiome studies to better understand the relationship between the microbial metagenome, metatranscriptome and disease.

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4. Gut microbiome contributes to impairment of immunity in pulmonary tuberculosis patients by alteration of butyrate and propionate producers.
   Maji A, Misra R, Dhakan DB, Gupta V, Mahato NK, Saxena R, *et al.*
   Environmental Microbiology. 20(1). 2018 January.
   PubMed ID: 29322681

ABSTRACT
Tuberculosis (TB) is primarily associated with decline in immune health status. As gut microbiome (GM) is implicated in the regulation of host immunity and metabolism, here we investigate GM alteration in TB patients by 16S rRNA gene and whole-genome shotgun sequencing. The study group constituted of patients with pulmonary TB and their healthy household contacts as controls (HCs). Significant alteration of microbial taxonomic and functional capacity was observed in patients with active TB as compared to the HCs. We observed that Prevotella and Bifidobacterium abundance were associated with HCs, whereas butyrate and propionate-producing bacteria like Faecalibacterium, Roseburia, Eubacterium and Phascolarctobacterium were significantly enriched in TB patients. Functional analysis showed reduced biosynthesis of vitamins and amino acids in favour of enriched metabolism of butyrate and propionate in TB subjects. The TB subjects were also investigated during the course of treatment, to analyse the variation of GM. Although perturbation in microbial composition was still evident after a month's administration of anti-TB drugs, significant changes were observed in metagenome gene pool that pointed towards recovery in functional capacity. Therefore, the findings from this pilot study suggest that microbial dysbiosis may contribute to pathophysiology of TB by enhancing the anti-inflammatory milieu in the host.

DOI: 10.1111/1462-2920.14015
IMPACT FACTOR: 5.39
CITED HALF-LIFE: 6.7

START COMMENTARY: This case-control study sought to examine the relationship between gut microbiota and TB infection by comparing differences in gut microbiota and microbial transcription between 6 TB patients and their household controls. Six cases were identified and fecal samples were collected before they began standard DOTS regimen. Additional fecal samples were collected one week and one month after the treatment began. Each case was matched to one control who was a blood-relative living in the same household and matched as closely on age as possible. The gut microbiota was examined in all fecal samples collected from cases and one sample from each control using 16S rRNA sequencing. Table 3 shows that the alpha diversity was higher in TB patients at all time points compared to the controls, but there were not significant differences in TB patients before and after starting treatment. Figure 2 further shows the differences in microbial populations between the TB subjects and healthy controls, and Figure 4 shows the functional differences associated with the differing microbial populations. The observed differences in microbial functional pathways related to flagellar assembly and vitamin/amino acid metabolism appeared to decrease in TB patients after a month of treatment. While this study is limited by its small sample size and short time series of samples from TB patients, it suggests there is an association between the gut microbiota and TB that should be further explored. In addition to the small sample size, the study has a potential bias in the selection of controls because they were not permitted to test controls for TB to confirm their negative status.

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5. Infant Gut Microbiome Associated With Cognitive Development.
   Carlson AL, Xia K, Azcarate-Peril MA, Goldman BD, Ahn M, Styner MA, *et al*.
   Biological Psychiatry. 83. 2018 January 15.
   PubMed ID: 28793975

ABSTRACT
BACKGROUND: Studies in rodents provide compelling evidence that microorganisms inhabiting the gut influence neurodevelopment. In particular, experimental manipulations that alter intestinal microbiota impact exploratory and communicative behaviors and cognitive performance. In humans, the first years of life are a dynamic time in gut colonization and brain development, but little is known about the relationship between these two processes.
METHODS: We tested whether microbial composition at 1 year of age is associated with cognitive outcomes using the Mullen Scales of Early Learning and with global and regional brain volumes using structural magnetic resonance imaging at 1 and 2 years of age. Fecal samples were collected from 89 typically developing 1-year-olds. 16S ribosomal RNA amplicon sequencing was used for identification and relative quantification of bacterial taxa.
RESULTS: Cluster analysis identified 3 groups of infants defined by their bacterial composition. Mullen scores at 2 years of age differed significantly between clusters. In addition, higher alpha diversity was associated with lower scores on the overall composite score, visual reception scale, and expressive language scale at 2 years of age. Exploratory analyses of neuroimaging data suggest the gut microbiome has minimal effects on regional brain volumes at 1 and 2 years of age.
CONCLUSIONS: This is the first study to demonstrate associations between the gut microbiota and cognition in human infants. As such, it represents an essential first step in translating animal data into the clinic.

DOI: 10.1016/j.biopsych.2017.06.021
IMPACT FACTOR: 11.41
CITED HALF-LIFE: 8.6

START COMMENTARY: The authors analyzed stool samples of infants enrolled in two longitudinal studies in North Carolina. Figure 1B shows the distribution of the three microbiota cluster groups defined in the study, characterized by the relatively high abundance of *Faecalibacterium* (Cluster 1), *Bacteroides* (Cluster 2), or Ruminococcaceae (Cluster 3). In addition, Cluster 1 showed the greatest alpha diversity, and Cluster 2 showed the least. Contrary to the authors' original hypothesis, greater microbiota diversity was negatively correlated with cognitive measures, and as such, infants with Cluster 2 compositions at age 1 year had the poorest cognitive outcomes at age 2 years. As shown in Table 1, there was a significant association between mode of delivery and microbiota cluster; almost 80% of infants in Cluster 2 were vaginally delivered. The results of this study support a growing field of literature that suggests a diverse microbiota is not always beneficial to all aspects of health. A major limitation of the study is the lack of a second stool sample at age 2 from most patients. Single samples were collected at age 1, an age where other studies have shown that the microbiota often changes rapidly. The lack of an additional stool sample at year 2 limits the authors' ability to confirm that those clusters identified at year 1 that correlated with intellectual development 1 year later, persisted at year 2. The study would be strengthened if the authors could show whether the microbial diversity and/or composition in these infants had changed between year 1 and 2.

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6. Genomic diversity and distribution of *Bifidobacterium longum* subsp. *longum* across the human lifespan.
   Odamaki T, Bottacini F, Kato K, Mitsuyama E, Yoshida K, Horigome A, *et al.*
   Scientific Reports. 8. 2018 January 08.
   PubMed ID: 29311585

ABSTRACT
Bifidobacterium longum subsp. *longum* represents one of the most prevalent bifidobacterial species in the infant, adult and elderly (human) gut. In the current study, we performed a comparative genome analysis involving 145 B. *longum* representatives, including 113 B. *longum* subsp. *longum* strains obtained from healthy Japanese subjects aged between 0 and 98 years. Although MCL clustering did not reveal any correlation between isolated strains and subject age, certain characteristics appear to be more prevalent among strains corresponding to specific host ages, such as genes involved in carbohydrate metabolism and environmental response. Remarkably, a substantial number of strains appeared to have been transmitted across family members, a phenomenon that was shown not to be confined to mother-infant pairs. This suggests that the ubiquitous distribution of B. *longum* subsp. *longum* across the human lifespan is at least partly due to extensive transmission between relatives. Our findings form a foundation for future research aimed at unraveling the mechanisms that allow B. *longum* strains to successfully transfer between human hosts, where they then colonize and persist in the gut environment throughout the host's lifespan.

DOI: 10.1038/s41598-017-18391-x
IMPACT FACTOR: 4.26
CITED HALF-LIFE: 2.0

START COMMENTARY: Authors evaluated stool samples from 453 subjects obtained in a previous cross-sectional study. They performed a microbiota analysis using 16S rRNA sequencing, isolation of *B. longum* subsp. *longum* strains by PCR in 177 samples, and genome sequencing of the stool samples. They then performed comparative genomics analysis to identify core genes and dispensable genes present in samples and used the core gene families to run a phylogenetic analysis. The results of the study concur with current literature that suggests the microbiota is stable over time following establishment during infancy. However, as shown in Figure 3, there is differential gene expression by age. Locus maps of the gene clusters enriched in younger subjects and older subjects are presented in Figures 4 and 5, respectively. There were 86 fecal samples obtained from subjects with at least one other family member in the study, representing 22 families. Analysis of the microbiota strains within family members suggested possible within family transmission in 12 of the 22 families. In five families, the same strain was found in all family members enrolled in the study. Supplementary Figure S5 shows the results of the mauve whole nucleotide alignment performed to assess the similarity between strains in family members. The study design limits the authors' ability to determine if the observed family transmission was vertical, between mother and child, or horizontal, between family members through shared space, and the direction of transmission. The cross-sectional study design also limited the authors' ability to look at changes in the microbiota overtime.

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7. Gene-trait matching across the Bifidobacterium longum pan-genome reveals considerable diversity in carbohydrate catabolism among human infant strains.
   Arboleya S, Bottacini F, O'Connell-Motherway M, Ryan CA, Ross RP, van Sinderen D, Stanton C.
   BMC Genomics. 19(1). 2018 January 08.
   PubMed ID: 29310579

ABSTRACT
BACKGROUND: *Bifidobacterium longum* is a common member of the human gut microbiota and is frequently present at high numbers in the gut microbiota of humans throughout life, thus indicative of a close symbiotic host-microbe relationship. Different mechanisms may be responsible for the high competitiveness of this taxon in its human host to allow stable establishment in the complex and dynamic intestinal microbiota environment. The objective of this study was to assess the genetic and metabolic diversity in a set of 20 *B. longum* strains, most of which had previously been isolated from infants, by performing whole genome sequencing and comparative analysis, and to analyse their carbohydrate utilization abilities using a gene-trait matching approach.
RESULTS: We analysed their pan-genome and their phylogenetic relatedness. All strains clustered in the *B. longum* ssp. *longum* phylogenetic subgroup, except for one individual strain which was found to cluster in the *B. longum* ssp. *suis* phylogenetic group. The examined strains exhibit genomic diversity, while they also varied in their sugar utilization profiles. This allowed us to perform a gene-trait matching exercise enabling the identification of five gene clusters involved in the utilization of xylo-oligosaccharides, arabinan, arabinoxylan, galactan and fucosyllactose, the latter of which is an abundant human milk oligosaccharide (HMO).
CONCLUSIONS: The results showed high diversity in terms of genes and predicted glycosyl-hydrolases, as well as the ability to metabolize a large range of sugars. Moreover, we corroborate the capability of *B. longum* ssp. *longum* to metabolise HMOs. Ultimately, their intraspecific genomic diversity and the ability to consume a wide assortment of carbohydrates, ranging from plant-derived carbohydrates to HMOs, may provide an explanation for the competitive advantage and persistence of *B. longum* in the human gut microbiome.

DOI: 10.1186/s12864-017-4388-9
IMPACT FACTOR: 3.73
CITED HALF-LIFE: 4.5

START COMMENTARY: Discovery of the *Bifidobacterium* genus is ongoing, and new species and subspecies are continually being discovered. One species in particular, *B. longum* has been shown to persist in high abundance in both infants and adults, but the particular strains of the species appear to differ between individuals. This analysis provides insight into the genome of 20 *B. longum* isolates from infants. As shown in Table 1, infants varied in age, mode of delivery and feeding source, all aspects that have been associated with differing microbiota composition. The authors determined a core genome of 1200 gene families that were common in all 20 isolates, representing 33% of the genes present. Figure 3C provides a detailed heat map of the core and variable genomes identified. Assuming these core genes are present in a larger population of samples, they could be used for targeted analysis in the future. The authors also compared the genomes sequenced from their samples with the current pan-genome for *B. longum* and found 20 newly sequenced genomes in their sample. Figure 2 shows an asymptotic trend between the number of genomes added and the number of genes in the pan-genome, suggesting that while currently open, after the inclusion of results from this study, the pan-genome of *B. longum* species is almost closed. Functional analysis of *B. longum* strains showed that several isolates can utilize or degrade fucosylated HMOs and plant derived carbohydrates. {Return to List of Articles}

8. The effect of probiotics and zinc supplementation on the immune response to oral rotavirus vaccine: A randomized, factorial design, placebo-controlled study among Indian infants.
Lazarus RP, John J, Shanmugasundaram E, Rajan AK, Thiagarajan S, Giri S, *et al.*
Vaccine. 36(2). 2018 January 04.
PubMed ID: 28874323

ABSTRACT
BACKGROUND: Strategies are needed to improve oral rotavirus vaccine (RV), which provides suboptimal protection in developing countries. Probiotics and zinc supplementation could improve RV immunogenicity by altering the intestinal microbiota and immune function.
METHODS: Infants 5 weeks old living in urban Vellore, India were enrolled in a randomized, double-blind, placebo-controlled trial with a 4-arm factorial design to assess the effects of daily zinc (5mg), probiotic (*1010Lactobacillus rhamnosus* GG) or placebo on the immunogenicity of two doses of RV (Rotarix®, GlaxoSmithKline Biologicals) given at 6 and 10 weeks of age. Infants were eligible for participation if healthy, available for the study duration and without prior receipt of RV or oral poliovirus vaccine other than the birth dose. The primary outcome was seroconversion to rotavirus at 14weeks of age based on detection of VP6-specific IgA at ≥20U/ml in previously seronegative infants or a fourfold rise in concentration.
RESULTS: The study took place during July 2012 to February 2013. 620 infants were randomized equally between study arms and 551 (88.9%) completed per protocol. Seroconversion was recorded in 54/137 (39.4%), 42/136 (30.9%), 40/143 (28.0%), and 37/135 (27.4%) infants receiving (1) probiotic and zinc, (2) probiotic and placebo, (3) placebo and zinc, (4) two placebos. Seroconversion showed a modest improvement among infants receiving probiotic (difference between groups 1, 2 and 3, 4 was 7.5% (97.5% Confidence Interval (CI): -1.4%, 16.2%), p=0.066) but not zinc (difference between groups 1, 3 and 2, 4 was 4.4% (97.5% CI: -4.4%, 13.2%), p=0.272). 16 serious adverse events were recorded, none related to study interventions.
CONCLUSIONS: Zinc or probiotic supplementation did not significantly improve the low immunogenicity of rotavirus vaccine given to infants in a poor urban community in India. A modest effect of combined supplementation deserves further investigation.
TRIAL REGISTRATION: The trial was registered in India (CTRI/2012/05/002677).

DOI: 10.1016/j.vaccine.2017.07.116
IMPACT FACTOR: 3.24
CITED HALF-LIFE: 6.0

START COMMENTARY: In addition to the primary analysis of seroconversion, the investigators explored several secondary outcomes to better elucidate the relationship between the interventions and vaccine immune response. A subset of 288 infants, selected among all arms, provided stool samples to evaluate rotavirus shedding and microbiota composition. Rotavirus shedding was highly correlated with RV seroconversion. 210 fecal samples were analyzed for *Lactobacillus,* and infants who took probiotics had significantly higher levels than those in the placebo arms. However, presence of *Lactobacillus* was not correlated with RV seroconversion. In addition to receiving the oral RV under study, infants in all four study arms also received routine immunizations during the study period, including oral polio virus (OPV). Analysis of seroconversion to OPV between study arms showed no significant difference in rates, with high seroconversion in all arms. Overall, the RV seroconversion rates seen in the study population were lower than those seen in previous studies in a similar population. The authors suggested the co-administration of OPV is the most likely cause of the decreased efficacy. Further studies are warranted

to determine if the modest association between probiotics and RV seroconversion observed in this study would be strengthened if RV is administered solo or if alternative probiotic strains are used.

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9. Evaluation of sampling and storage procedures on preserving the community structure of stool microbiota: A simple at-home toilet-paper collection method.
   Al KF, Bisanz J, Gloor GB, Reid G, Burton JP.
   Journal of Microbiological Methods. 144. 2018 January.
   PubMed ID: 29155236

ABSTRACT
BACKGROUND: The increasing interest on the impact of the gut microbiota on health and disease has resulted in multiple human microbiome-related studies emerging. However, multiple sampling methods are being used, making cross-comparison of results difficult. To avoid additional clinic visits and increase patient recruitment to these studies, there is the potential to utilize at-home stool sampling. The aim of this pilot study was to compare simple self-sampling collection and storage methods.
METHODS: To simulate storage conditions, stool samples from three volunteers were freshly collected, placed on toilet tissue, and stored at four temperatures (-80, 7, 22 and 37°C), either dry or in the presence of a stabilization agent (RNAlater®) for 3 or 7 days. Using 16S rRNA gene sequencing by Illumina, the effect of storage variations for each sample was compared to a reference community from fresh, unstored counterparts. Fastq files may be accessed in the NCBI Sequence Read Archive: Bioproject ID PRJNA418287.
RESULTS: Microbial diversity and composition were not significantly altered by any storage method. Samples were always separable based on participant, regardless of storage method suggesting there was no need for sample preservation by a stabilization agent.
DISCUSSION: In summary, if immediate sample processing is not feasible, short term storage of unpreserved stool samples on toilet paper offers a reliable way to assess the microbiota composition by 16S rRNA gene sequencing.

DOI: 10.1016/j.mimet.2017.11.014
IMPACT FACTOR: 1.79
CITED HALF-LIFE: 9.5

START COMMENTARY: Most field studies with fecal samples use clinic collected stool samples or at home collected stool samples that are frozen after collection or sent to the laboratory in a short amount of time where they can be frozen before analysis. This technique is of limited value in the field and limits sites selection to those close to laboratories or with self-storage. In 2015, the American Gut Project developed a simple test in which participants swab a small amount of fecal material from a stool or soiled toilet paper sample and mail it to the laboratory without refrigeration. This technique is most likely preferable in field studies, but the possibility of sample degradation with this sample and storage technique has been questioned. Some researchers have suggested adding a stabilizing agent to reduce degradation. This study examined the microbiota composition in stool samples from three individuals collected using different techniques and stored at different temperatures for different amounts of time before analysis. Figure 2 shows the results of the analysis, where independent of the storage temperature, time, or presence of a preservation agent, the microbiota of each participant clustered together and accurately identified the patient. This suggests that samples can be collected at home and stored at room temperature up to 7 days before processing and still provide useful results. This study is limited by its small sample size. Additionally, samples were collected in a sterile environment, and the temperature was kept constant over the time period. These two conditions may not always be met in the field, and a similar study should be replicated under field conditions.

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10. Immunogenicity of rotavirus vaccine (Rotarix™) in infants with environmental enteric dysfunction.
Mwape I, Bosomprah S, Mwaba J, Mwila-Kazimbaya K, Laban NM, Chisenga CC, *et al*.
PLoS One. 12(12). 2017 December 27.
PubMed ID: 29281659

ABSTRACT
INTRODUCTION: Deployment of rotavirus vaccines has contributed to significant declines in diarrheal morbidity and mortality globally. Unfortunately, vaccine performance in low-middle income countries (LMICs) is generally lower than in developed countries. The cause for this has been associated with several host and maternal factors including poor water sanitation and hygiene (WASH) status, which are predominant in LMICs. More recently, environmental enteric dysfunction (EED) has specifically been hypothesized to contribute to poor vaccine uptake and response. The aim of this study was to examine the association between serological biomarkers of EED and seroconversion to rotavirus vaccine in Zambian infants.
METHODS: This was a retrospective cohort study of 142 infants who had been fully immunized with Rotarix™, and had known seroconversion status. Seroconversion was defined as 4-fold or more increase in rotavirus-specific IgA titres between pre-vaccination and one month post-dose two vaccination. We performed ELISA assays to assess soluble CD14 (sCD14), Endotoxin Core IgG Antibodies (EndoCAb), intestinal fatty acid binding protein (i-FABP) and Zonulin according to the manufacturers protocols. Generalised linear model with family-poisson, link-log and robust standard error was used to estimate the independent effects of biomarkers on seroconversion adjusting for important cofounders.
RESULTS: The median concentration of Zonulin, Soluble CD14, EndoCaB, and IFABP were 209.3 (IQR = 39.7, 395.1), 21.5 (IQR = 21.5, 21.5), 0.3 (IQR = 0.3, 0.3), and 107.7 (IQR = 6.4, 1141.4) respectively. In multivariable analyses adjusting for the independent effect of other biomarkers and confounders (i.e. age of child at vaccination, breast-milk anti-rotavirus IgA, infant serum anti-rotavirus IgG, and IgA seropositivity at baseline), there was strong evidence of about 24% increase in seroconversion due to doubling Zonulin concentration (Adjusted risk ratio (aRR) = 1.24; 95% CI = 1.12 to1.37; p<0.0001). Similarly, we found about 7% increase in seroconversion due to doubling IFABP concentration (aRR = 1.07; 95% CI = 1.02 to 1.13; p = 0.006).
CONCLUSION: We found that high levels of zonulin and IFABP played a role in seroconversion. It is plausible that increased gut permeability in EED allows greater uptake of the live virus within the vaccine, but later consequences result in deleterious local structural distortions and malabsorption syndromes.

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START COMMENTARY: Authors analyzed EED biomarkers in pre-vaccine sera samples from infants who received Rotarix™ vaccine between 6 to 12 weeks of age and had a known seroconversion status (134 seroconverters and 83 non-seroconverters). The study suggests a relationship between EED status and Rotarix effectiveness that should be further explored, as both this study and a prior study in Bangladesh (Uddin, 2016) reported positive associations between EED biomarkers and seroconversion following oral vaccination (in the prior study, oral cholera vaccine was used). The study has several limitations. Investigators did not assess any stool biomarkers of EED and did not examine infant microbiota composition or activity at the time of vaccination. Additionally, authors did not collect longitudinal stool

or blood samples to evaluate possible associations over time between EED, the microbiota and seroconversion.

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