Clinical and Experimental Gastroenterology

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

Why targeting the microbiome is not so successful: can randomness overcome the adaptation that occurs following gut manipulation?

This article was published in the following Dove Press journal:
Clinical and Experimental Gastroenterology

Yaron Ilan

Department of Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Abstract: The microbiome is explored as a potential target for therapy of bowel and systemic diseases. Fecal microbiota transplantation (FMT) has demonstrated efficacy in Clostridium difficile infection. However, clinical results regarding other diseases are modest, despite the abundant research on the microbiome over the last decade. Both high rate variability of the microbiome and adaptation to gut manipulations may underlie the lack of ultimate effects of FMT, probiotics, prebiotics, synbiotics, and antibiotics, which are aimed at restoring a healthier microbiome. The present review discusses the inherent variability of the microbiome and multiple factors that affect its diversity, as possible causes of the adaptation of the gut microbiome to chronic manipulation. The potential use of randomness is proposed, as a means of overcoming the adaptation and of restoring some of the inherent variability, with the goal of improving the long-term efficacy of these therapies.

Keywords: microbiome, randomness, gut, fecal transplantation

Introduction

The microbiome is suggested as a potential target for treatment of gut-related or microbiota-related bowel and systemic diseases. Fecal microbiota transplantation (FMT) has demonstrated efficacy for *Clostridium difficile* infection (CDI).[1] Clinical results for most other diseases are modest, though research of the microbiome has flourished over the last decade. Microbiota-based therapies for other disorders than CDI are performed only in research settings.[2] The targeting of FMT to several potential diseases is reviewed herein, along with mechanisms that may explain the moderate success and failure of the procedures. Adaptation of the gut microbiome to its manipulation by FMT or antibiotics, probiotics, prebiotics, synbiotics, and phage therapy are discussed. Standardized microbiota replacement therapies should be based on the understanding of both the mechanisms of action and safety of these therapies. The use of randomness as a means of overcoming microbiome adaptation, restoring part of its inherent variability, and potentially altering the gut–brain axis are proposed for improving the efficacy of these procedures.

Correspondence: Yaron Ilan
Department of Medicine, Hebrew University-Hadassah Medical Center, Ein-Kerem, POB 1200, Jerusalem IL91120, Israel
Tel +972 2 677 8231
Email ilan@hadassah.org.il

The complex interactions between the microbiome and the host

The complex interactions between gut microbiota involve the role of the host and the microbiota, including microbiota metabolites, in host protection against

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/CEG.S203823

pathogens, regulating host physiological functions that comprise metabolism, and developing and maintaining the balance between the immune system and the nervous system.[3–5] The healthy human microbiota in the gut is highly diverse comprising between 500 and 2,000 species.[2] Metagenomic carriage of metabolic pathways was stable among subjects despite variation in community structure and racial background.[4,5]

Dysbiosis, the gut microbial imbalance, leads to dysfunction of host machineries, which underlies and contributes to the pathogenesis of numerous diseases.[6] Dysbiosis is associated with several intestinal disorders, including celiac disease, inflammatory bowel disease, and irritable bowel syndrome (IBS). It is also connected to extraintestinal diseases including cardiovascular disorders, allergy, obesity, asthma, cancer, and sepsis.[6] While the debate is ongoing regarding the impact of dysbiosis on the progression of these disorders, recent data support a more complex connection which is not a simple cause-and-effect relationship.[3] Both inherent variability of the microbiome and adaptation to manipulations are difficulties faced in the attempt to restore a healthier microbiome. Table 1 summarizes some of the difficulties associated with FMT.

FMT is used for the treatment of CDI, but variable results have been achieved for other indications

FMT increases the recipient's gut microbiome diversity and restores microbial balance homeostasis, and is thereby thought to alleviate dysbiosis-associated symptoms.[7] FMT is effective in the management of CDI. The recurrence rate of CDI is 20%. A review of seven clinical trials for treatment of multiple recurrent CDI with FMT showed efficacy of this mode of therapy in this setting.[8] However, FMT is not currently endorsed for use outside of CDI. Both efficacy and safety concerns were raised with regard to its use in other disorders.[7]

Positive results of the clinical efficacy of FMT other than for CDI have involved the treatment ulcerative colitis (UC).[7] Randomized controlled trials showed it can induce both clinical and endoscopic remission in active UC patients.[9–12] A recent analysis of 18 studies including 446 UC patients showed efficacy compared to placebos, with a low risk of heterogeneity. Colonoscopy delivery and the use of unrelated fecal donor slightly improved the results of FMT treatment.[13] Failure to achieve consistent clinically meaningful findings has been attributed to technical discrepancies between methods.[14] A trend for positive outcomes in Crohn's disease (CD) has been observed in small studies.[15,16] In both UC and CD, microbiota exploration following FMT revealed augmented microbiota diversity and a shift toward the donor bacterial profile in recipients' stools. However, the microbiomes were followed for a relatively short time, and therefore, the possibility of subsequent adaptation or recurrence of the "sick" microbiome was not determined. Duration, frequency, route of administration, and donor selection are some additional variables that have been suggested to determine the efficacy of FMT.[9,17,18]

IBS is thought to be triggered by bacterial, viral, or parasitic infections. The composition of the microbiome in IBS varies from that of healthy subjects and is consistent with dysbiosis in the intestinal microbiome.[19] Targeting the microbiota for treatment of IBS, via the use of FMT, as well as by nonabsorbable antibiotics, prebiotics, probiotics, and synbiotics, was tested in small studies.[20] In some

Table 1 Factors that impact studies of fecal microbiota transplantation and their clinical efficacy

Host related parameters	Gender Age Diet Body weight Concomitant disease and medications
Microbiome-related parameters	Being a highly dynamic and constantly changing organ Rapid adaptation to manipulation
Environmental factors	The response to exposomes: environmental factors that the host interacts with Household contacts
Testing	Software used for analyzing data Exploring feces vs scraping the bowel wall itself for microbiome analysis

studies, FMT reversed the dysbiosis to normobiosis and reduced the clinical signs in 70% of treated subjects. An association between constipation and intestinal microbiota disturbance suggested the possibility of targeting the microbiome in chronic functional constipation using probiotics, prebiotics, synbiotics, or FMT. Microbial treatment was shown to improve clinical symptoms and promote the recovery of intestinal microbiota.[21] The majority of studies on this topic looked at the severity scores as endpoints. Inclusion criteria differed between the trials, as did the means of FMT administration. The inconsistency between studies makes reaching conclusive results difficult.[22] Moreover, the long-term effects of FMT on bowel diversity were not analyzed; thus, the possibility remains of some degree of adaptation and re-dysbiosis.

Numerous studies have proposed a part for the gut microbiome in the development of autoimmune diseases. A modification of the microbiome (lower Firmicutes/Bacteroidetes ratio) was described in patients with systemic lupus erythematosus. Alterations in the intestinal bacteria and periodontal disease were suggested to contribute to the pathogenesis of asthma, systemic sclerosis, rheumatoid arthritis, Sjogren's syndrome, and antiphospholipid syndrome.[23,24] Dysbiosis was also suggested to increase vulnerability to sepsis and to its associated complications. Recovery of the intestinal bacteria was suggested to be protective.[25] Altered microbiota signatures were described in colorectal cancer (CRC) patients. Adenoma was associated with increased diversity, while CRC with a decrease. *Escherichia coli*, *Fusobacterium nucleatum*, and enterotoxigenic *Bacteroides fragilis* are increased in cancerous tissues. Presenting certain bacterial strains CRC models in mice supports a possible association. Enterotoxins, genotoxins, and virulence factors derived from gut microbiome were related to bacteria-driven tumorigenesis.[26]

Overall, the evidence suggests an association of dysbiosis with systemic diseases, while attempts to manipulate the microbiome are still far from being successful. Most of the diseases described above are chronic. Re-dysbiosis and adaptation of the microbiome to change may explain, at least in part, the lack of long-term efficacy of FMT. A microbial ecosystem rich in Bacteroidetes and Proteobacteria and low in Clostridium clusters IV and XIVa was noted in UC patients after FMT and was predictive of poor sustained response. Additionally, sustained response was associated with restoration of the butyrate production capacity.[27] Improved fecal microbiota preparation reduced the rates of adverse effect, but did not affect the clinical efficacy in patients with CD.[15]

A high degree of variability and randomness in gut microbiome

Data from several studies showed that the microbiome is characterized by a high degree of variability and randomness. Genome sequencing was performed on 178 microbiome-derived genomes. From a total of 547,968 predicted polypeptides which match the gene complement of these strains, unidentified ones were recognized. These unidentified polypeptides revealed sequences and that did not match any nonreference record in the nonredundant subset. The study yielded a set of 30,867 polypeptides, of which 97% were distinctive. Analyzing this platform of microbial genomes showed that 40% of random sequences from the gut bacteria were related to organisms according to the criteria used.[28] Similarly, the microbiome is needed for the catabolism of dietary fibers since the human genome does not encode carbohydrate-active enzymes. Genetic analysis isolated 310 clones that showed activities of galactanase, amylase, hemicellulase, beta-glucanase, or pectinase. Sequencing of nonredundant metagenomic DNA, matching to 26 clones effective for raw plant polysaccharides metabolism, identified 73 carbohydrate-active enzymes from 35 families. These corresponded to a fivefold target-gene augmentation when matched with random sequencing of gut metagenome, 33 of which were homologous to predominant genes found in the microbiome of 20 subjects for whom metagenomic data are available.[29] This leaves a substantial proportion unidentified. The data suggest that while some random sequences can be associated with organisms, considerable variability and randomness exist, which are hard to control. This randomness may be inherent in the microbiome, and is an obstacle that needs to be overcome when manipulating the gut by FMT. The relatively low degree of adherence between repeated tests of the microbiome further supports the existence of intrinsic variability, which may also explain the high degree of discrepancies between studies.

Analysis of the T-cell amino acid motif repertoires of the proteomes of two groups of microbiome-derived bacteria showed that 3.2 million of these motifs bind to T-cell receptors. An overlap in motif usage was documented. The proteome comprises only three-quarters of the conceivable motifs, of which 65% are part of the in microbiota proteomes. Immunoglobulin variable regions show a wide

variety of T-cell exposed motifs (TCEMs). It established a random sample of motifs that are found in the proteome from the microbiome, human proteome, and pathogens. Discrete bacterial species differ in their immunoglobulins and human proteome matched motifs which they express. Several bacteria preserve a motif signature with the human proteome pattern.[30] The high rate of variability in motifs has an impact on attempts at systemic immunomodulation via manipulation of the microbiome. Moreover, differentiating self from non-self is not individual TCEM dependent. It depends on dynamic signals in which similar TCEM displays dissimilar roles in different organisms. The rate by which a specific TCEM exerts its influence may also differ.[30]

Shifts in the oral microbiome after the initiation of antiretroviral therapy in HIV patients contribute to immune phenomena and inflammatory disease. High variability in bacterial communities was demonstrated both within participants and between time points. At baseline, the number of taxa and the alpha diversity was variable, but did not differ significantly based on viral load, CD4, or other features. After 24 weeks of antiretroviral therapy, patients with low CD4 counts had higher microbiota diversity. Some variances with periodontal illness were described, manifesting differences between baseline and post-treatment states.[31] The inter- as well as intrapatient variability further complicates attempts to develop consistent methods for reducing dysbiosis by means of FMT.

Longitudinal changes in gut microbiome

Recent data and modeling support a high degree of variability in the microbiome at any given point of time and also high variability that transpires over time. Longitudinal studies of the gut microbiome are identifying the bacteria associated with clinical consequences or with environmental parameters. Some intestinal microbiota compositional data are skewed. Results from repeated measures in these studies do not always correlate. Most gut bacteria studies use unrelated samples and are inappropriate for longitudinal and pedigree studies. Ignoring such correlations may bias conclusions. A two-part zero-inflated beta regression model with random impacts determined an association between microbiota types and clinical manifestation over prolonged follow-up. A logistic regression model was used for determining the presence ofcertain bacteria in the samples. A beta regression model was used for displaying the nonzero microbiota, where each component includes a random effect; this accounts for the associations between recurrent measurements on the same patient. The model enabled studying the taxa based on longitudinal or recurrent measures.[32] In another study, the correlated sequence kernel association test was applied to study these relationships using a linear mixed model. Random effects accounted for the outcome connections, and a difference component test tested the impact of the microbiome. This method better calibrated the null distribution of the score test statistic to match the relatively minor sample size.[33] Overall, these methods highlight the importance of considering both the variability at any point of time and long-term variability, withregard to alterations of the microbiome.

External factors impact variability in the microbiome

Multiple factors contribute to microbiome variability, further complicating attempts to manipulate it. Prebiotic supplementation affects gut microbiota structure and function. A microbiome analysis was undertaken, based on the early intestinal microbiome information for foreseeing the difference in Bifidobacterium after prebiotic intake. A short-term prebiotic intervention decreased the alpha-diversity of the intestinal microbiome. Fecal sample analysis revealed that a fructo-oligosaccharide (FOS) intervention reduced the alpha diversity of the microbiome, which rebounded on day 9. Treatment with galacto-oligosaccharides (GOS) decreased intestinal alpha diversity during treatment. Neither FOS nor GOS altered beta diversity of gut microbiota.[34] Diets can also alter the microbiome. Choline intake affects the level of trimethylamine N-oxide (TMAO) breakdown by the microbiome in humans. Beta diversity of the gut microbiota was found to differ between humans with increased and decreased plasma TMAO levels following a choline challenge.[35] Microbial communities and metabolome vary significantly between dogs fed a Bones and Raw Food (BARF) diet and commercially fed dogs. The BARF group was fed higher quantities of protein and fat and lower amounts of nitrogen-free extract and fiber. Linear discriminant analysis effect size displayed higher abundances of Fusobacterium, Clostridium, Lactobacillales, and Enterobacteriaceae in the BARF group, while conservatively fed dogs showed an increase of Ruminococcaceae, Clostridiaceae, Erysipelotrichaceae, and Lachnospiraceae. Random forest analysis showed increased 4-aminobutyric acid and 4-hydroxybutryric acid in the BARF group.[36]

Background diseases can alter the microbiome. In the shift from cirrhosis to early hepatocellular carcinoma, fecal microbial diversity was found to increase; increases were particularly observed in Gemmiger formicilis, Parabacteroides, and Phylum Actinobacteria, and in genera producing-lipopolysaccharide. In parallel, butyrate-producing genera were found to decrease.[37] A shift in intestinal microbiome in response to infection of white spot syndrome virus (WSSV) was shown at 6 and 48 hrs postinfection. Four phyla (Bacteroidetes, Firmicutes, Tenericutes, and Proteobacteria) were abundant in the intestine of *E. sinensis*, irrespective of the stage of the WSSV infection. Further analysis revealed significant differences in abundances of over 12 bacterial phyla, 44 orders, and 68 families at numerous states of the infection. The data suggest that variations in intestine microbiota are associated with the degree of severity of WSSV infection.[38]

Ecological principles affect animal-associated microbiota. The relevance of selection and random processes in the generation of gut bacteria was studied. Intestinal bacterial composition was related to the host phylogeny and diet. The amount of bacterial lineages per intestine sample correlated with animal mass, such that larger animals have bigger numbers of anaerobes.[39] Studies of membrane biofilm groups in membrane bioreactors described the mature biofilm communities. A recent study investigated whether the assembly of biofilm communities comes from random immigration of species from the source community. Alpha and beta diversity was shown to differ in microbiome community structure between samples of activated sludge (AS) and samples of early and mature biofilm in the membrane bioreactors. The changes were due to the occurrence of higher numbers of rare operational taxonomic units (OTUs). Although there was a large portion of shared sequence between AS and biofilm samples, simulated biofilm communities obtained from random sampling of the respective AS community discovered that biofilm communities varied from the random assemblies.[40] While these data indicate that the biofilm communities do not fully signify a random sample of the AS community, some degree of variability is apparent, which is determined by environmental factors.[40] Blastocystis subtypes were derived from shotgun metagenomics and implemented to 12 datasets, comprising 1,689 humans of various geographic sources, disease status, and lifestyle. A nonrandom and ST-specific distribution was shown, along with an ability to cause persistent colonization. Lack of correlation with any of the diseases considered implied that Blastocystis is part of a healthy intestinal microbiota. The profiles of 43 Blastocystis genomes demonstrated increased intrasubtype variability of ST1 and ST2 than with ST3 and ST4.[41] The high rate of mutagenesis further supports a high degree of variability, which results from environmental changes.

Exercise also affects diversity. Microbiome variability and alterations in inflammatory indicators related to exercise were determined in mice that performed voluntary exercise or moderate forced exercise. Intestinal gut microbiota profiling compared the OTUs in samples. A multivariate analysis of beta diversity via Adonis testing did not recognize major changes in patterns compared to sedentary control mice. A random forest machine learning model which accounts for relationships between bacteria in a community, categorized voluntary exercisers, and non-exercisers, with 97% accuracy. The common bacteria the model used were known taxa (S24-7, Bacteroides, and Lactobacillus) and novel taxa (Lachnospiraceae and Rikenellaceae) that were associated with exercise.[42]

Taken together, these data support multiple external factors, as well as inherent variability, as determinants of the degree of diversity and dysbiosis of the microbiome.

Gut microbiome is a vital part of the gut–brain network, which further determines a high degree of variability in the microbiome

The microbiome develops synchronously with the brain. Evidence shows that the microbiome may affect normal mental processes and may trigger several mental and neurological diseases. The microbiota was suggested as a potential therapeutic target.[43] Dysbiosis and changes in bacteria-derived metabolites were described in Parkinson's disease. Some of these metabolites are associated with intestine inflammation and neuroinflammation.[44] Autism spectrum disorder is associated with dysbiosis and with a reciprocal interaction network between the microbiome, gut, and brain.[45] The neonate is exposed to the maternal vaginal microbiome, which is a basis for normal intestine colonization and contributes to immune maturation. These host–microbiome connections occur during neurodevelopment, thus supporting the hypothesis of early crosstalk between the developing intestine and brain. Alterations in the vaginal microbiome may induce changes in offspring intestine microbiome and also in the brain. Vaginal immunity and the richness of Lactobacillus, the abundant taxa in

the vagina, are associated with the maternal stress-altered proteins. A decrease of the vaginal Lactobacillus is associated with reduction its transmission to the offspring. Alterations in the structure of the microbiome in the neonate gut resembled the change patterns of metabolism which play a role in energy balance, and in disruptions of amino acid profiles in the developing brain.[46] A case of FMT in the case of CD which led to amelioration of epilepsy was described.[47] These data suggest that bidirectional interactions of the brain–microbiome may be relevant to the re-dysbiosis process that may occur following therapies.

The models used may affect the results of microbiome testing

While the inherent variability in the microbiome is affected by multiple factors, the specific models used also affect results. Studies suggested incomplete support for the hypothesis that changes in the microbiota are related to obesity.[48] A recent analysis of 10 studies demonstrated associations of alpha diversity metrics with the hazard of obesity for only a limited number of parameters. When the data were pooled using a random-effects model, correlations were noted for Shannon diversity, number of OTUs, Shannon evenness, and obesity. Associations were not detected for the ratio of Bacteroidetes and Firmicutes, or their distinct abundances. A power analysis confirmed a single study had sufficient power to detect a 5% change in diversity. Random forest machine learning models showed that the median accuracy varied between 33% and 64%. Although some support was demonstrated for an association between the human microbiome and obesity status, this correlation was weak and was confounded by high interpersonal dissimilarity and relatively small sample sizes.[48]

Next-generation sequencing characterizes microbial community composition. The microbiome taxonomic profile was studied using shotgun analysis of random DNA fragments or via using 16S ribosomal RNA gene (rDNA) amplicon sequencing. The 16S sequencing approach resulted in quantitatively and qualitatively dissimilar results than those of the shotgun-derived data. The shotgun sequencing metagenomics enabled better representation of microbiome complexity and identified a greater number of species for an individual sample than 16S rDNA amplicon sequencing.[49] Similarly, high-throughput sequencing technology is used for determining bacterial counts and the composition of taxa at different taxonomic levels of the microbiome. The correlation of specific taxon with phenotypes has been assessed using a linear mixed-effect model. This modeling integrates phylogenetic information among bacterial communities. Alternatively, models that consider all taxa attain assortment via the penalization method, which disregards phylogenetic data. A regression analysis using taxa at diverse levels, showed that for individual taxon. A kernel matrix was designed based on distance measures in the phylogenetic tree which functions as a variance component in the model. Taxonomic selection is then attained using the lasso (least absolute shrinkage and selection operator) penalty on variance components. This method showed better performance in a long-term gut microbiota study of HIV-infected patients.[50] Metagenomic sequencing data are affected by the diverse total sequence delivered across samples, by overdispersion and zero inflation, and by the collection of samples with hierarchical structures. All these generate correlations among the samples, thus complicating the examination. A negative binomial mixed model was proposed for determining the relationship between the gut microbiota and host clinical parameters. This mixed-effects model accounted for correlations between the samples by integrating random effects into the fixed-effects negative binomial model and was able to deal with the overdispersion and variance in total reads.[51] Collection, storage, DNA extraction, and next-generation sequencing technologies impact the results.[52] These examples show that the models being used may affect microbiome analysis.

Overcoming microbiome variability by introducing more randomness

The microbiome is a highly dynamic organ with its own individually tailored order. The high degree of variability in microbiome diversity and composition appears to be due to a combination of inherent randomness and multiple factors that affect the gut bacteria. Many of these factors are hard to control. Attempts to change the microbiome are challenged by the induction of an "artificial order" into a highly variable system. This may lead to a phase of adaptation and re-dysbiosis. In clinical practice, the effect of microbiome manipulation may subside. An attempt to "reorder the disorder" via induction of a "continuous order" by probiotics, antibiotics, synbiotics, or FMT may be unsuccessful due to the lack of consideration of the ongoing adaptation toward the therapy, the personalized dynamic determined by host and external parameters, and the inherent variability of the microbiome. Improving FMT by selective microbiota

transplantation and by using newer strategies and methodologies was suggested.[53,54]

Randomness was suggested to be inherent in various biological systems[55] and was proposed as a means of improving the efficacy of therapies.[56,57] The problem of inducing an "artificial order" into an inherent disordered system may be overcome by redisordering. This may enable resolving adaptation and tolerance to therapies such as FMTs and lead to better long-term responses. Randomness can be achieved by altering the type of bacteria used, employing multiple or alternating donors, and by interchanging between different manipulations of the gut microbiome.

In summary, the microbiome is likely to become a target for therapy of systemic diseases. The inherent variability of the microbiome, and the multiple host- and environment-derived factors that affect dysbiosis, should be addressed by improving the methods implemented. Elucidating the inherent microbiome variability may enable the design of randomness-based gut manipulations that can overcome adaptation to therapy and the re-dysbiosis phenomenon, thus enabling improved long-term effects of these treatments.

Abbreviations List

FMT, fecal microbiota transplantation; CDI, clostridium difficile infection; UC, ulcerative colitis; CD, Crohn's disease; IBS, irritable bowel syndrome; CRC, colorectal cancer; TCEMs, T-cell exposed motifs; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides; TMAO, trimethylamine N-oxide; BARF, bones and raw food; AS, activated sludge; OTUs, operational taxonomic units; WSSV, white spot syndrome virus.

Disclosure

Dr Yaron Ilan is the Chief scientific officer at Oberon, and a consultant for Teva; ENZO; Protalix; Betalin Therapeutics; Immuron; SciM; Natural Shield; Oberon Sciences; Tiziana Pharma; Plantylight; Exalenz Bioscience. Dr Yaron Ilan also reports a patent for use of randomness in medicine pending. The author reports no other conflicts of interest in this work.

References

1. Baktash A, Terveer EM, Zwittink RD, et al. Mechanistic insights in the success of fecal microbiota transplants for the treatment of clostridium difficile infections. *Front Microbiol*. 2018;9:1242. doi:10.3389/fmicb.2018.01242
2. Khanna S. Microbiota replacement therapies: innovation in gastrointestinal care. *Clin Pharmacol Ther*. 2018;103:102–111. doi:10.1002/cpt.923
3. Kho ZY, Lal SK. The human gut microbiome - a potential controller of wellness and disease. *Front Microbiol*. 2018;9:1835. doi:10.3389/fmicb.2018.01835
4. Human Microbiome Project C. A framework for human microbiome research. *Nature*. 2012;486:215–221. doi:10.1038/nature11209
5. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486:207–214. doi:10.1038/nature11234
6. Gagliardi A, Totino V, Cacciotti F, et al. Rebuilding the gut microbiota ecosystem. *Int J Environ Res Public Health*. 2018;15(8):pii: E1679. doi:10.3390/ijerph15081679.
7. Holleran G, Scaldaferri F, Ianiro G, et al. Fecal microbiota transplantation for the treatment of patients with ulcerative colitis and other gastrointestinal conditions beyond clostridium difficile infection: an update. *Drugs Today (Barc)*. 2018;54:123–136. doi:10.1358/dot.2018.54.2.2760765
8. Ooijevaar RE, van Beurden YH, Terveer EM, et al. Update of treatment algorithms for clostridium difficile infection. *Clin Microbiol Infect*. 2018;24:452–462. doi:10.1016/j.cmi.2017.12.022
9. Jeon SR, Chai J, Kim C, Lee CH. Current evidence for the management of inflammatory bowel diseases using fecal microbiota transplantation. *Curr Infect Dis Rep*. 2018;20:21. doi:10.1007/s11908-018-0627-8
10. Costello SP, Hughes PA, Waters O, et al. Effect of fecal microbiota transplantation on 8-week remission in patients with ulcerative colitis: a randomized clinical trial. *JAMA*. 2019;321:156–164. doi:10.1001/jama.2018.20046
11. Paramsothy S, Nielsen S, Kamm MA, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. *Gastroenterology*. 2019;156(5):1440.e2–1454.e2. doi:10.1053/j.gastro.2018.12.001.
12. Paramsothy S, Kamm MA, Kaakoush NO, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet*. 2017;389:1218–1228. doi:10.1016/S0140-6736(17)30182-4
13. Cao Y, Zhang B, Wu Y, Wang Q, Wang J, Shen F. The value of fecal microbiota transplantation in the treatment of ulcerative colitis patients: a systematic review and meta-Analysis. *Gastroenterol Res Pract*. 2018;2018:5480961. doi:10.1155/2018/5480961
14. Fairhurst NG, Travis SPL. Why is it so difficult to evaluate faecal microbiota transplantation as a treatment for ulcerative colitis? *Intest Res*. 2018;16:209–215. doi:10.5217/ir.2018.16.2.209
15. Wang H, Cui B, Li Q, et al. The safety of fecal microbiota transplantation for Crohn's disease: findings from a long-term study. *Adv Ther*. 2018;35:1935–1944. doi:10.1007/s12325-018-0800-3
16. He Z, Li P, Zhu J, et al. Multiple fresh fecal microbiota transplants induces and maintains clinical remission in Crohn's disease complicated with inflammatory mass. *Sci Rep*. 2017;7:4753. doi:10.1038/s41598-017-04984-z
17. D'Odorico I, Di Bella S, Monticelli J, Giacobbe DR, Boldock E, Luzzati R. Role of fecal microbiota transplantation in inflammatory bowel disease. *J Dig Dis*. 2018;19:322–334. doi:10.1111/1751-2980.12603
18. Matijasic M, Mestrovic T, Peric M, et al. Modulating composition and metabolic activity of the gut microbiota in IBD patients. *Int J Mol Sci*. 2016;17(4): pii:E578. doi:10.3390/ijms17040578.
19. El-Salhy M, Mazzawi T. Fecal microbiota transplantation for managing irritable bowel syndrome. *Expert Rev Gastroenterol Hepatol*. 2018;12:439–445. doi:10.1080/17474124.2018.1447380
20. Rodino-Janeiro BK, Vicario M, Alonso-Cotoner C, Pascua-García R, Santos J. A review of microbiota and irritable bowel syndrome: future in therapies. *Adv Ther*. 2018;35:289–310. doi:10.1007/s12325-018-0673-5
21. Huang L, Zhu Q, Qu X, Qin H. Microbial treatment in chronic constipation. *Sci China Life Sci*. 2018;61:744–752. doi:10.1007/s11427-017-9220-7
22. Schmulson M, Bashashati M. Fecal microbiota transfer for bowel disorders: efficacy or hype? *Curr Opin Pharmacol*. 2018;43:72–80. doi:10.1016/j.coph.2018.08.012

23. De Luca F, Shoenfeld Y. The microbiome in autoimmune diseases. *Clin Exp Immunol*. 2019;195(1):74–85. doi:10.1111/cei.13158.
24. Kang Y, Cai Y. Future prospect of faecal microbiota transplantation as a potential therapy in asthma. *Allergol Immunopathol (Madr)*. 2018;46:307–309. doi:10.1016/j.aller.2017.04.008
25. Haak BW, Prescott HC, Wiersinga WJ. Therapeutic potential of the gut microbiota in the prevention and treatment of sepsis. *Front Immunol*. 2018;9:2042. doi:10.3389/fimmu.2018.02042
26. Yu LC, Wei SC, Ni YH. Impact of microbiota in colorectal carcinogenesis: lessons from experimental models. *Intest Res*. 2018;16:346–357. doi:10.5217/ir.2018.16.3.346
27. Fuentes S, Rossen NG, van der Spek MJ, et al. Microbial shifts and signatures of long-term remission in ulcerative colitis after faecal microbiota transplantation. *ISME J*. 2017;11:1877–1889. doi:10.1038/ismej.2017.44
28. Human Microbiome Jumpstart Reference Strains C, Nelson KE, Weinstock GM, et al. A catalog of reference genomes from the human microbiome. *Science*. 2010;328:994–999. doi:10.1126/science.1183605
29. Tasse L, Bercovici J, Pizzut-Serin S, et al. Functional metagenomics to mine the human gut microbiome for dietary fiber catabolic enzymes. *Genome Res*. 2010;20:1605–1612. doi:10.1101/gr.108332.110
30. Bremel RD, Homan EJ. Extensive T-cell epitope repertoire sharing among human proteome, gastrointestinal microbiome, and pathogenic bacteria: implications for the definition of self. *Front Immunol*. 2015;6:538. doi:10.3389/fimmu.2015.00538
31. Presti RM, Handley S, Droit L, et al. Alterations in the oral microbiome in HIV-infected participants after ART administration are influenced by immune status. *Aids*. 2018;32:1279–1287. doi:10.1097/QAD.0000000000001811
32. Chen EZ, Li H. A two-part mixed-effects model for analyzing longitudinal microbiome compositional data. *Bioinformatics*. 2016;32:2611–2617. doi:10.1093/bioinformatics/btw308
33. Zhan X, Xue L, Zheng H, et al. A small-sample kernel association test for correlated data with application to microbiome association studies. *Genet Epidemiol*. 2018. doi:10.1002/gepi.22160
34. Luo YM, Liu FT, Chen MX, et al. [A machine learning model based on initial gut microbiome data for predicting changes of Bifidobacterium after prebiotics consumption]. *Nan Fang Yi Ke Da Xue Xue Bao*. 2018;38:251–260.
35. Lu JQ, Wang S, Yin J, et al. [A machine learning model using gut microbiome data for predicting changes of trimethylamine-N-oxide in healthy volunteers after choline consumption]. *Nan Fang Yi Ke Da Xue Xue Bao*. 2017;37:290–295.
36. Schmidt M, Unterer S, Suchodolski JS, et al. The fecal microbiome and metabolome differs between dogs fed Bones and Raw Food (BARF) diets and dogs fed commercial diets. *PLoS One*. 2018;13:e0201279. doi:10.1371/journal.pone.0201279
37. Ren Z, Li A, Jiang J, et al. Gut microbiome analysis as a tool towards targeted non-invasive biomarkers for early hepatocellular carcinoma. *Gut*. 2018. doi:10.1136/gutjnl-2017-315084
38. Ding ZF, Cao MJ, Zhu XS, Xu GH, Wang RL. Changes in the gut microbiome of the Chinese mitten crab (Eriocheir sinensis) in response to white spot syndrome virus (WSSV) infection. *J Fish Dis*. 2017;40:1561–1571. doi:10.1111/jfd.12624
39. Sherrill-Mix S, McCormick K, Lauder A, et al. Allometry and ecology of the bilaterian gut microbiome. *MBio*. 2018;9(2):pii:e00319–18. doi:10.1128/mBio.00319-18.
40. Matar GK, Bagchi S, Zhang K, Oerther DB, Saikaly PE. Membrane biofilm communities in full-scale membrane bioreactors are not randomly assembled and consist of a core microbiome. *Water Res*. 2017;123:124–133. doi:10.1016/j.watres.2017.06.052
41. Beghini F, Pasolli E, Truong TD, Putignani L, Cacciò SM, Segata N. Large-scale comparative metagenomics of blastocystis, a common member of the human gut microbiome. *ISME J*. 2017;11:2848–2863. doi:10.1038/ismej.2017.139
42. Lamoureux EV, Grandy SA, Langille MGI. Moderate exercise has limited but distinguishable effects on the mouse microbiome. *mSystems*. 2017;2(4):pii:e00006–e00017. doi:10.1128/mSystems.00006-17.
43. McIlroy J, Ianiro G, Mukhopadhya I, Hansen R, Hold GL. Review article: the gut microbiome in inflammatory bowel disease-avenues for microbial management. *Aliment Pharmacol Ther*. 2018;47:26–42. doi:10.1111/apt.14384
44. Sun MF, Shen YQ. Dysbiosis of gut microbiota and microbial metabolites in Parkinson's disease. *Ageing Res Rev*. 2018;45:53–61. doi:10.1016/j.arr.2018.04.004
45. Yang Y, Tian J, Yang B. Targeting gut microbiome: A novel and potential therapy for autism. *Life Sci*. 2018;194:111–119. doi:10.1016/j.lfs.2017.12.027
46. Jasarevic E, Howerton CL, Howard CD, Bale TL. Alterations in the vaginal microbiome by maternal stress are associated with metabolic reprogramming of the offspring gut and brain. *Endocrinology*. 2015;156:3265–3276. doi:10.1210/en.2015-1177
47. He Z, Cui BT, Zhang T, et al. Fecal microbiota transplantation cured epilepsy in a case with Crohn's disease: the first report. *World J Gastroenterol*. 2017;23:3565–3568. doi:10.3748/wjg.v23.i19.3565
48. Sze MA, Schloss PD. Looking for a signal in the noise: revisiting obesity and the microbiome. *MBio*. 2016;7(4):pii:e01018–e01216. doi:10.1128/mBio.01018-16.
49. Laudadio I, Fulci V, Palone F, Stronati L, Cucchiara S, Carissimi C. Quantitative assessment of shotgun metagenomics and 16S rDNA amplicon sequencing in the study of human gut microbiome. *OMICS*. 2018;22:248–254. doi:10.1089/omi.2018.0013
50. Zhai J, Kim J, Knox KS, Twigg HL, Zhou H, Zhou JJ. Variance component selection with applications to microbiome taxonomic data. *Front Microbiol*. 2018;9:509. doi:10.3389/fmicb.2018.00509
51. Zhang X, Mallick H, Tang Z, et al. Negative binomial mixed models for analyzing microbiome count data. *BMC Bioinformatics*. 2017;18:4. doi:10.1186/s12859-016-1441-7
52. Panek M, Cipcic Paljetak H, Baresic A, et al. Methodology challenges in studying human gut microbiota - effects of collection, storage, DNA extraction and next generation sequencing technologies. *Sci Rep*. 2018;8:5143. doi:10.1038/s41598-018-23296-4
53. Zhang F, Cui B, He X, Nie Y, Wu K, Fan D. Microbiota transplantation: concept, methodology and strategy for its modernization. *Protein Cell*. 2018;9:462–473. doi:10.1007/s13238-018-0541-8
54. Chu ND, Smith MB, Perrotta AR, Kassam Z, Alm EJ, Zoetendal EG. Profiling living bacteria informs preparation of fecal microbiota transplantations. *PLoS One*. 2017;12:e0170922. doi:10.1371/journal.pone.0170922
55. Ernst G. Hidden signals-the history and methods of heart rate variability. *Front Public Health*. 2017;5:265. doi:10.3389/fpubh.2017.00081
56. Kyriazis M. Editorial: novel approaches to an old problem: insights, theory and practice for eliminating aging. *Curr Aging Sci*. 2014;7:1–2.
57. Kyriazis M. Practical applications of chaos theory to the modulation of human ageing: nature prefers chaos to regularity. *Biogerontology*. 2003;4:75–90.