Published in final edited form as:
J Allergy Clin Immunol. 2012 May ; 129(5): 1204–1208. doi:10.1016/j.jaci.2012.03.010.

The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites

Luke K Ursell, B.S.[1], **Jose C Clemente, Ph.D.**[1], **Jai Ram Rideout**[2], **Dirk Gevers, Ph.D.**[3], **J Gregory Caporaso, Ph.D.**[2], and **Rob Knight, Ph.D.**[1,4,*]

[1]Department of Chemistry and Biochemistry, University of Colorado at Boulder, Boulder, CO, 80309, USA

[2]Department of Computer Science, Northern Arizona University, Flagstaff, AZ, 86011, USA

[3]Microbial Systems and Communities, Genome Sequencing and Analysis Program, Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

[4]Howard Hughes Medical Institute, Boulder, CO, 80309, USA

Abstract

The human body harbors 10–100 trillion microbes, mainly bacteria in our gut, which greatly outnumber our own human cells. This bacterial assemblage, referred to as the human microbiota, plays a fundamental role in our well-being. Deviations from healthy microbial compositions (dysbioses) have been linked with important human diseases, including inflammation-linked disorders such as allergies, obesity and inflammatory bowel disease. Characterizing the temporal variations and community membership of the healthy human microbiome is critical in order to accurately identify the significant deviations from normality that could be associated with disease states. However, the diversity of the human microbiome varies between body sites, between individuals, and over time. Environmental differences have also been shown to play a role in shaping the human microbiome in different cultures, requiring that the healthy human microbiome be characterized across lifespans, ethnicities, nationalities, cultures, and geographic locales. In this paper, we summarize our knowledge on the microbial composition of the five best-characterized body sites (gut, skin, oral, airways, and vagina), focusing on inter- and intrapersonal variations and our current understanding of the sources of this variation.

Keywords

human microbiome; microbial diversity; temporal variation; 16S rRNA sequencing

*Corresponding Author Rob Knight rob.knight@colorado.edu Howard Hughes Medical Institute University of Colorado at Boulder Department of Chemistry and Biochemistry 215 UCB Boulder, CO 80309-0215 USA +1-303-492-7506 administration +1-303-492-5894 (FAX).
Jai Ram Rideout is currently an undergraduate student in the Department of Computer Science at Northern Arizona University

Introduction

The human body is colonized by trillions of microbial cells, collectively referred to as the microbiota, while the combination of these microbial cells and their corresponding suite of genes is defined as the microbiome (1). The introduction of sample barcoding (2–4), the decreasing cost of next-gen sequencing technologies (5, 6), improvements in bioinformatics tools (7, 8), and online databases (9–11) has allowed researchers to categorize what microbes live in and on the human body, and to define the similarities and differences between human microbiota. The first human-associated microbial studies quickly discovered the high degree of variability in the microbiota between individuals (12–16); these studies were rapidly extended to show that variability is also high within individuals both between different body sites and over time within one body site (17–19). More recent studies have been able to sample the microbiota densely over time (20) and in large cohorts (21). Efforts like the Human Microbiome Project (1, 21, 22) are beginning to elucidate the variations found in healthy adult microbial communities. It is uncertain at this point whether the differences in microbiota seen in many disease states are a symptom of the disease or a contributing factor. However, defining a healthy microbial state is a critical step for discovering how variations in the microbiome may contribute to or cause a wide range of diseases (23).

Techniques for microbial community analysis

Sample barcoding coupled with high-throughput sequencing has allowed microbiologists to study microbial communities at an unprecedented depth over the past few years. By identifying each sample with a unique nucleotide barcode added on to the PCR primer used to amplify microbial 16S ribosomal RNA, samples can be pooled together and run at the same time on a high-throughput sequencer. The sequences can then be imported into a number of software pipelines for microbial analysis, including mothur (24), W.A.T.E.R.S (25), and QIIME (7). The prevalence of cloud computing, including the Amazon Web Serivces (AWS) Elastic Compute Cloud (EC2), means that anyone with internet access can connect to a supercomputer and analyze hundreds of millions of microbial sequences with minimal up-front costs (e.g., renting a computer with 8 processors and 68GB of RAM from AWS costs about USD$2.00 per hour).

The open-source software QIIME (Quantitative Insights Into Microbial Ecology; www.qiime.org; pronounced “chime”) takes users from their raw sequence data, through quality filtering and other initial analysis steps, through alpha and beta diversity analyses (defined below), and ultimately through publication quality graphics. An early step in microbial community analysis workflows is clustering of sequence reads into operational taxonomic units, or OTUs. An OTU cluster is usually defined on the basis of sequence similarity: frequently reads that are greater than or equal to 97% identical to one another are clustered into an OTU. This is primarily done for computational efficiency: compute-intensive downstream steps, such as assigning taxonomy to sequences, can be performed on a single representative sequence from an OTU rather than on many nearly identical sequences.

Ultimately, many researchers are interested in understanding the microbial diversity of their samples: main alpha (or within-sample) diversity, and beta (or between-sample) diversity. Alpha diversity might be measured, for example, as the number of unique operational taxonomic units (OTUs) found wtihin a given community. Beta diversity, on the other hand, is frequently measured by computing pairwise UniFrac distances, (the fraction of branch length in a phylogenetic tree that is unique to either sample (26)) between samples. Communities that are very similar phylogenetically result in low UniFrac scores, while dissimilar communities produce high UniFrac scores. UniFrac distances between many

samples can be represented in a distance matrix, and that distance matrix can be summarized and visualized in 3-dimensional space using principal coordinates analysis (PCoA), a dimensionality reduction technique that summarizes the distances between samples in a scatterplot where points (representing samples) that are more distant from one another are more dissimilar.

Gut Microbiota

Impact of diet in defining gut microbial communities

The human gut represents an important reservoir of bacteria that has been shown to play an important role in human health, including priming the host immune system and possibly causing disease states through microbial community dysbiosis (27, 28). Diet is the most powerful influence on gut microbial communities in healthy humans (29–32). A study of humans and 59 other mammals revealed clustering where the effects of diet (carnivory, omnivory, or herbivory) in most cases outweighed host phylogeny (30). Recent analysis suggested that the gut microbiota could be classified as belonging to one of three principal variants, or "enterotypes", defined by a dominant presence of *Bacteroides, Prevotella*, or *Ruminococcus* (31). However, these enterotypes seem to be more microbial gradients than discrete communities, and can largely be explained by long-term dietary intake: *Bacteroides* was prevalent with long-term protein and animal fat diets, while *Prevotella* was associated with long-term carbohydrate diets (32).

Twin studies have also been influential in elucidating the role that environment plays in defining the gut microbiome. One study compared monozygotic and dizygotic twins living in South Korea and the United States, including pairs of European and African descent (33). Alpha diversity was not significantly different between the Korean and US cohorts demonstrating that one cohort did not contain a greater number of OTUs than the other. UniFrac distances between the two groups revealed that the phylogenetic composition of the gut community in the Korean cohort was significantly different from the US cohort (including the African American and European American subgroups). Family level taxa that discriminated between the Korean and US cohorts included Bacteroidaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae, Prevotellaceae, Rikenellaceae, Ruminococaceae, Streptococcaceae, and *Veillonellaceae* (33).

Altered microbiota in obese individuals

Differences between the South Korean and US cohorts decreased when comparing obese individuals across the two groups (33). PCoA revealed that distinct clustering of South Korean and US cohorts were greater when comparing only lean individuals than when comparing lean and obese individuals. This suggests that obesity is masking some of the dietary and environmental factors between these two groups. Twin studies reveal that one difference between the gut communities of lean and obese individuals might be related to reduced alpha diversity, which is commonly seen in obese patients relative to lean patients (17, 33). Obesity has also been observed to correlate with several phylum-level bacterial changes, including decreased *Bacteroidetes* and increased *Firmicutes* within subjects on a weight-reduction diet (34), although the pattern between subjects in lean and obese populations appears to be more complex (17, 35, 36).

Biogeography of human gastrointestinal tract (GIT)

Recent studies have evaluated human-associated microbiota along the length of the gastrointestinal tract. Work by Stearns et al. sampled the mouth, stomach, duodenum, colon and stool from 2 healthy males and 2 healthy females. They found that the mouth harbored the greatest phylogenetic diversity, the stomach had the lowest diversity, and diversity

increased down the GIT from the stomach to the stool (37). Twenty-five OTU's at various taxonomic levels were present in every sampling site of every individual, including *Faecalibacterium*, TM7, and *Streptococcus*. As is typical in human microbiota studies, clustering was seen between sample sites along the GIT rather than clustering based on subject or gender (37).

Interestingly, constrained ordinations methods have been used to demonstrate that differences exist between male and female microbial communities (38). In this study, males (n=5) clustered more closely together than females (n=5), and were enriched in *Faecalibacterium prausnitzii, Bifidobacterium* spp, *Bacteroides*, *Clostridium*, *Enterococcus*, and *Prevotella*. Females had enhanced signals from *Streptococcus*, *Veillonella*, *Mannheimia* and *Ruminococcus* spp relative to males (38). The GIT has also been shown to have a biogeographical distribution of microbes. Using numerical ecology methods to remove inter-subject variability, one study suggests that there might be evidence of microbial gradients along the GIT. For example, *Enterobacteriaceae* was shown to increase towards the distal end of the GIT (the sigmoid colon and rectum), while *Streptococcus*, *Comamonadaceae*, *Enterococcus*, and *Corynebacterium* had increased abundance in the proximal end of the GIT (the caecum and transverse colon) (38).

Skin Microbiota

The skin represents an interesting human habitat where lifestyle and environmental factors shape the microbial community of different specific body sites. No taxa are ubiquitously present in every individual and body site, though targeted studies reveal that specific body sites are generally dominated by certain defining taxa: *Bacteroidetes* in the GI tract, *Lactobacillus* in the vagina, *Streptococcus* in the oral cavity, and *Propionibacterium* in retroauricular crease (16–19, 22, 39). The human skin is mainly comprised of Actinobacteria, Proteobacteria, and Firmicutes, with one study finding that more than 90% of the microbiota of the forearm belonged to these phyla (40).

A hallmark of human skin microbiota communities is high diversity and high interpersonal variation. Costello et al. found that skin sites including the palms, fingers, and forearm had greater phylogenetic diversity than communities in the gut, external auditory canal, or oral cavity (19). The volar forearm of different individuals were found to only share 2% of species-level OTUs (40) while the hands share 13% of OTUs (14). Estimates of species-level OTUs for skin sites include 246 for the volar forearm (40), >150 for the palms of hands (14), and 113 for inner elbow (15). More than 50% of sequences obtained from arm skin sites belong to Propionibacteria, Corynebacteria, Staphylococcus, Streptococcus, and Lactobacillus (14, 40). Diversity among skin sites of the same individual is also high. One study found that of the total 48 species-level OTUs found on the forearms, on average only 13.5 were shared between the left and right forearms on the same individual, representing 67.9% of clones (40). Similarly, the left and right hands of the same individual were shown to share 17% of OTUs, with diversity more than threefold greater than the forearm or elbow (14). While the skin does harbor hundreds of unique OTUs, our current level of sequencing is likely not revealing all OTUs present (15).

Fungal microbiota of the human forearm have also been explored in healthy patients and in patients with psoriasis (41). Five healthy patients and three with psoriasis had their forearms sampled and the 18s rDNA was sequenced to detect eukaryotes. Most sequences obtained resembled the *Malassezia* at the genus level, and differences in the communities of psoriasis patients were noted in a majority of cases. One limitation to this study was the low level of 18s rDNA sequences present in GenBank at the time. As databases improve, our knowledge

of the presence and diversity of eukaryotic microbiota on the human body will continue to increase.

Oral Microbiota

The microbial community of the oral cavity is unique compared to other body habitats and contains high variability between individuals (19, 42–44). Different oral sites, including mucosal sites, anaerobic pockets, and teeth each harbor unique microbial assemblages (13, 46). In a study of the oral communities from 10 healthy adults, 15 bacterial genera were found in all individuals, including 10 species such as Streptococcus oralis, Haemophilus parainfluenzae, Granulicatella adiacens, Veillonella parvula, Veillonella dispar, Rothia aeria, Actinomyces naeslundii, Actinomyces odontolyticus, Prevotella melaninogenica, and *Capnocytophaga gingivalis*. Despite these similarities, the oral communities had high interpersonal variation with some oral communities a majority dominated by Streptococcus, while others were dominated by *Prevotella*, *Neisseria*, *Haemophilus*, or *Veillonella*. Many species were not shared between individuals for a given genera, including *Neisseria*, *Fusobacterium, and Corynebacterium* (42). Another study sampled the oral cavity of 120 individuals from 12 different geographical locations (43).

While no geographical patterns were apparent, large variations in microbial communities existed between locations. For example, 28% of all sequences derived from Congo were *Enterobacter*, yet this taxa was not found in China, Germany, Poland, Turkey, or California. Larger variations in the microbial communities were found between individuals in the same geographical location than between geographical locations, demonstrating that global geography does not seem to play a role in determining oral microbial communities. The number of genera present (defined as 90% sequence similarity) in any individual ranged from six to 30, and 39 previously unreported genera were identified within the human oral cavity (43).

Airway Microbiome

The microbiota associated with the airways has many parallels with other more highly studied parts of the human body. For instance, the distribution of bacteria within the lungs is spatially heterogeneous (47), exactly the same pattern witnessed in other human body parts including the skin (48). Also, distinct microbial communities are present in the airways of diseased patients, including cystic fibrosis and asthma (47, 49). For example, both culturing and deep sequencing have revealed that *Pseudomonasaeruginosa*, *Staphyloccus aureus*, and *Burkholderia cepacia* are presented in the lungs of patients with cystic fibrosis (CF) (47). In CF patients *P. aeruginosa* can comprise up to 99% of bacterial sequences isolated from the trachea and 51%–94% of sequences from each lobe of the lung. It has been shown that age can also have a significant impact on the airway microbiota of CF patients. Using 16s rRNA PhyloChip to assess the microbiota of patients from nine to 72 years of age, the study found that older CF patients had a decrease in bacterial richness, evenness, and diversity while concurrently loosing pulmonary function (50). It would seem that, in CF patients at least, the highly diverse young airway microbiota is gradually replaced by a less diverse community where a few members, including *Pseudomonas* spp., *Staphylococcus* spp., and *Burkholderia* spp. are highly dominant.

The airway microbiota has also been shown to play a role in asthma. A comparison of 65 asthma patients on inhaled corticosteroids with 10 health controls found that asthmatic patients' airways contained a greater microbial diversity than healthy controls (51). Approximately 100 bacterial phyla whose presence was highly correlated with bronchial hyperresponsiveness were also identified, including the families *Sphingomonadaceae*, *Oxalobacteraceae*, *Comamonadaceae*, *Enterobacteriaceae*, and *Shewanellaceae* (51). In a

large adult cohort study, it was found that the prevalence of asthma was negatively correlated with the presence of Helicobacter pylori (52). One hypothesis for the development of asthma postulates that exposure to diverse microbes early in life may have a protective effect against asthma (53).

Vaginal Microbiome

The vaginal microbial community has long been considered an important defense mechanism against infection (54–56). Studies that sampled women across different ethnicities including Caucasian, African American, Hispanic, and Asian found that most vaginal communities could be defined by the presence of a dominating *Lactobacillus* species of *L. iners, L. crispatus, L. gasseri*, or *L. jensenii* (16, 57). The other communities were not dominated by a *Lactobacillus* species, but still contained a dominant community of lactic-acid producing microbes (16). The vaginal communities of Asian and Caucasian women were most often dominated by lactic-acid producing *Lactobacillus* than Hispanic and African American women, possibly causing the lower vaginal pH levels found in Asian and Caucasian women. Bacterial vaginosis (BV) results in a significant community shift from healthy communities and negative health consequences (16). Twenty nine percent of species-level OTUs were shared between healthy and BV-positive women, as BV-positive communities were characterized by decreases in Lactobacillus and increases in Gardnerella, Atopobium, Megasphaera, Eggerthella, Aerococcus, Leptotrichia/Sneathia, Prevotella and *Papillibacter* (58).

Temporal Variation of Human Microbiome Diversity

Development of the human microbiome is a dynamic process, with different life stages displaying notable differences in terms of diversity and variation (59). Variation between human body sites in adults is stable over time, but different body sites converge on the healthy adult microbiome via different trajectories. For example, newborns are rapidly colonized by the microbial communities of the mother's vagina if delivered vaginally, or by microbes resembling skin if delivered via cesarean (60). The child's gut microbiota acquires phylogenetic diversity linearly, resembling a healthy adult by two years of age (61). However, child oral communities do not resemble adults' even at 18 years (44). The reasons for these differences in colonization are not yet known.

Once developed, stable differences were observed between human body sites over three months (19). Dense sampling over time answered more specific questions about the degree and scale of temporal variation. Caporaso et al (20) studied two individuals sampled daily at four body sites (tongue, left and right palms, and gut): one female for 6 months, and one male for 18 months. Variation was greatest in skin communities, followed by gut communities; oral communities were most stable. Interpersonal differences in community composition within body sites were also stable across time. Next, the authors show that despite stability over time, there is a relatively small `temporal core microbiome' at the 97% OTU level. In other words, while communities look relatively similar over time, there are few OTUs that are actually observed at all time points. The size of this `temporal core microbiome' at the species level correlates with variability: the oral communities have the largest core (approximately 10% of the OTUs are present in 95% of the samples), the gut communities have the next largest core (approximately 5% of OTUs are present in 95% of the samples) and the skin communities have the smallest core (approximately 1% of OTUs are present in 95% of the samples). There appears to be no core temporal microbiome across body sites.

Conclusion

The decreasing cost of sequencing has allowed researchers to obtain an unprecedented quantity of 16S rRNA sequencing from larger cohorts sampled more densely over time. These large-scale sampling efforts have corroborated the results of numerous small studies in affirming the large interpersonal variation of the microbiota within a given body habitat, and the immense differences found between different body habitats. However, it is possible that some of our original hypothesis on the microbiota developed from small sample number experiments may be overturned as the trend of ever-larger cohorts continues. The sampling of new populations at increasing depth is continuing to find novel species-level OTUs, demonstrating how our complete characterization of human-associated microbes is not yet complete. These OTUs can be very important for determining differences between communities and in defining disease states. While the most recent wave of microbial studies focused on increasing the number of sequences and samples collected, the challenge facing future studies is to increase the clinically relevant information associated with samples to better relate changes in the microbiota to events in human lives. With the continued decrease in the cost of sequencing and the increasing accessibility of the necessary bioinformatics tools, we expect that our understanding of human-associated microbial communities will soon result in novel microbiome-related clinical treatments. We now know what “normal” communities look like to an unprecedented extent: what we need to discover, in a systematic way, is what “diseased” communities look like, and which factors can be manipulated in order to bring them back to the healthy state.

Abbreviations

OTU	Operational Taxonomic Unit
GIT	Gastrointestinal Tract
CF	Cystic Fibrosis
BV	Bacterial Vaginosis

References

1. Turnbaugh PJ, et al. The human microbiome project. Nature. 2007; 449(7164):804–810. [PubMed: 17943116]
2. Church GM, Kieffer-Higgins S. Multiplex DNA sequencing. Science. 1988; 240(4849):185–188. [PubMed: 3353714]
3. Huber JA, et al. Microbial population structures in the deep marine biosphere. Science. 2007; 318(5847):97–100. [PubMed: 17916733]
4. Hamady M, Walker JJ, Harris JK, Gold NJ, Knight R. Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. Nature Methods. 2008; 5(3):235–237. [PubMed: 18264105]
5. Caporaso JG, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(Suppl 1):4516–4522. [PubMed: 20534432]
6. Metzker ML. Sequencing technologies - the next generation. Nat Rev Genet. 2010; 11(1):31–46. [PubMed: 19997069]
7. Caporaso JG, et al. QIIME allows analysis of high-throughput community sequencing data. Nature Methods. 2010; 7(5):335–336. [PubMed: 20383131]
8. Glass EM, Wilkening J, Wilke A, Antonopoulos D, Meyer F. Using the metagenomics RAST server (MG-RAST) for analyzing shotgun metagenomes. Cold Spring Harb Protoc. 2010; 2010(1) pdb prot5368.

9. DeSantis TZ, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006; 72(7):5069–5072. [PubMed: 16820507]
10. Cole JR, et al. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 2009; 37(Database issue):D141–145. [PubMed: 19004872]
11. Pruesse E, et al. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 2007; 35(21):7188–7196. [PubMed: 17947321]
12. Eckburg PB, et al. Diversity of the human intestinal microbial flora. Science. 2005; 308(5728): 1635–1638. [PubMed: 15831718]
13. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005; 43(11):5721–5732. [PubMed: 16272510]
14. Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(46):17994–17999. [PubMed: 19004758]
15. Grice EA, et al. A diversity profile of the human skin microbiota. Genome Res. 2008; 18(7):1043–1050. [PubMed: 18502944]
16. Ravel J, et al. Vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(Suppl 1):4680–4687. [PubMed: 20534435]
17. Turnbaugh PJ, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457(7228): 480–484. [PubMed: 19043404]
18. Grice EA, et al. Topographical and temporal diversity of the human skin microbiome. Science. 2009; 324(5931):1190–1192. [PubMed: 19478181]
19. Costello EK, et al. Bacterial community variation in human body habitats across space and time. Science. 2009; 326(5960):1694–1697. [PubMed: 19892944]
20. Caporaso JG, et al. Moving pictures of the human microbiome. Genome Biol. 2011; 12(5):R50. [PubMed: 21624126]
21. Proctor LM. The Human Microbiome Project in 2011 and beyond. Cell Host & Microbe. 2011; 10(4):287–291. [PubMed: 22018227]
22. Peterson J, et al. The NIH Human Microbiome Project. Genome Res. 2009; 19(12):2317–2323. [PubMed: 19819907]
23. Young VB. The intestinal microbiota in health and disease. Curr Opin Gastroenterol. 201 1
24. Schloss PD, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009; 75(23):7537–7541. [PubMed: 19801464]
25. Hartman AL, Riddle S, McPhillips T, Ludascher B, Eisen JA. Introducing W.A.T.E.R.S.: a workflow for the alignment, taxonomy, and ecology of ribosomal sequences. BMC Bioinformatics. 2010; 11:317. [PubMed: 20540779]
26. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005; 71(12):8228–8235. [PubMed: 16332807]
27. Jarchum I, Pamer EG. Regulation of innate and adaptive immunity by the commensal microbiota. Curr Opin Immunol. 2011; 23(3):353–360. [PubMed: 21466955]
28. Ley RE. Obesity and the human microbiome. Curr Opin Gastroenterol. 2010; 26(1):5–11. [PubMed: 19901833]
29. Muegge BD, et al. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science. 2011; 332(6032):970–974. [PubMed: 21596990]
30. Ley RE, et al. Evolution of mammals and their gut microbes. Science. 2008; 320(5883):1647–1651. [PubMed: 18497261]
31. Arumugam M, et al. Enterotypes of the human gut microbiome. Nature. 2011; 473(7346):174–180. [PubMed: 21508958]
32. Wu GD, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011; 334(6052):105–108. [PubMed: 21885731]

33. Lee S, Sung J, Lee J, Ko G. Comparison of the gut microbiotas of healthy adult twins living in South Korea and the United States. Appl Environ Microbiol. 2011; 77(20):7433–7437. [PubMed: 21873488]
34. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444(7122):1022–1023. [PubMed: 17183309]
35. Kallus SJ, Brandt LJ. The Intestinal Microbiota and Obesity. J Clin Gastroenterol. 2011
36. Flint HJ. Obesity and the gut microbiota. J Clin Gastroenterol. 2011; 45(Suppl):S128–132. [PubMed: 21992951]
37. Stearns JC, et al. Bacterial biogeography of the human digestive tract. Sci. Rep. 2011; 1
38. Aguirre de Carcer D, et al. Numerical ecology validates a biogeographical distribution and gender-based effect on mucosa-associated bacteria along the human colon. The ISME journal. 2011; 5(5): 801–809. [PubMed: 21124491]
39. Qin J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010; 464(7285):59–65. [PubMed: 20203603]
40. Gao Z, Tseng CH, Pei Z, Blaser MJ. Molecular analysis of human forearm superficial skin bacterial biota. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104(8):2927–2932. [PubMed: 17293459]
41. Paulino LC, Tseng CH, Strober BE, Blaser MJ. Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. J Clin Microbiol. 2006; 44(8):2933–2941. [PubMed: 16891514]
42. Bik EM, et al. Bacterial diversity in the oral cavity of 10 healthy individuals. The ISME journal. 2010; 4(8):962–974. [PubMed: 20336157]
43. Nasidze I, Li J, Quinque D, Tang K, Stoneking M. Global diversity in the human salivary microbiome. Genome Res. 2009; 19(4):636–643. [PubMed: 19251737]
44. Crielaard W, et al. Exploring the oral microbiota of children at various developmental stages of their dentition in the relation to their oral health. BMC Med Genomics. 2011; 4:22. [PubMed: 21371338]
45. Keijser BJ, et al. Pyrosequencing analysis of the oral microflora of healthy adults. J Dent Res. 2008; 87(11):1016–1020. [PubMed: 18946007]
46. Zaura E, Keijser BJ, Huse SM, Crielaard W. Defining the healthy “core microbiome” of oral microbial communities. BMC Microbiol. 2009; 9:259. [PubMed: 20003481]
47. Willner D, et al. Spatial distribution of microbial communities in the cystic fibrosis lung. The ISME journal. 2012; 6(2):471–474. [PubMed: 21796216]
48. Costello EK, et al. Bacterial Community Variation in Human Body Habitats Across Space and Time. Science. 2009; 326(5960):1694–1697. [PubMed: 19892944]
49. Hilty M, et al. Disordered microbial communities in asthmatic airways. PLoS One. 2010; 5(1):e8578. [PubMed: 20052417]
50. Cox MJ, et al. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. PLoS One. 2010; 5(6):e11044. [PubMed: 20585638]
51. Huang YJ, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. J Allergy Clin Immunol. 2011; 127(2):372–381. e371–373. [PubMed: 21194740]
52. Reibman J, et al. Asthma is inversely associated with Helicobacter pylori status in an urban population. PLoS One. 2008; 3(12):e4060. [PubMed: 19112508]
53. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? Nat Rev Microbiol. 2009; 7(12):887–894. [PubMed: 19898491]
54. Donders GG, et al. Pathogenesis of abnormal vaginal bacterial flora. Am J Obstet Gynecol. 2000; 182(4):872–878. [PubMed: 10764465]
55. Gupta K, et al. Inverse association of H2O2-producing lactobacilli and vaginal Escherichia coli colonization in women with recurrent urinary tract infections. J Infect Dis. 1998; 178(2):446–450. [PubMed: 9697725]

56. Watts DH, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. J Infect Dis. 2005; 191(7):1129–1139. [PubMed: 15747249]

57. Zhou X, et al. Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women. The ISME journal. 2007; 1(2):121–133. [PubMed: 18043622]

58. Ling Z, et al. Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis. BMC Genomics. 2010; 11:488. [PubMed: 20819230]

59. Dominguez-Bello MG, Blaser MJ, Ley RE, Knight R. Development of the human gastrointestinal microbiota and insights from high-throughput sequencing. Gastroenterology. 2011; 140(6):1713–1719. [PubMed: 21530737]

60. Dominguez-Bello MG, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107(26):11971–11975. [PubMed: 20566857]

61. Koenig JE, et al. Succession of microbial consortia in the developing infant gut microbiome. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(Suppl 1):4578–4585. [PubMed: 20668239]