Review Article

Human microbial ecology and the rising new medicine

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Abstract: The first life forms on earth were Prokaryotic, and the evolution of all Eukaryotic life occurred with the help of bacteria. Animal-associated microbiota also includes members of the archaea, fungi, protists, and viruses. The genomes of this host-associated microbial life are called the microbiome. Across the mammalian tree, microbiomes guarantee the development of immunity, physiology, and resistance to pathogens. In humans, all surfaces and cavities are colonized by a microbiome, maintained by a careful balance between the host response and its colonizers—thus humans are considered now supraorganisms. These microbiomes supply essential ecosystem services that benefit health through homeostasis, and the loss of the indigenous microbiota leads to dysbiosis, which can have significant consequences to disease. This educational review aims to describe the importance of human microbial ecology, explain the ecological terms applied to the study of the human microbiome, developments within the cutting-edge microbiome field, and implications to diagnostic and treatment.

Keywords: Human microbiome; ecology; medicine

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A single origin in the evolution of the planets' microbiota

We live in a microbial world, and all life on earth should be framed in the context of microbial evolution. Briefly, early life on earth is thought to have evolved from a primordial soup, and there is evidence that life has evolved from a single origin (1). Australian stromatolites—considered the first evidence of microbial life—being laminated, fossilized microbial communities of the Precambrian-, are presumed to have performed sulfur-driven photosynthesis ~3.5-Ga via protocyanobacteria that used iron as reductant. Cyanobacteria producing oxygenic photosynthesis appeared ~2.8 Ga (2-4). Ever so early, our planet was dominated by unicellular microorganisms and their contributions to environmental evolution are enormous. Phylogenetic analyses reveal two prominent domains of life, the Bacteria and the Archaea with the eukaryotes emerging from the latter (5). This view of eukaryotes being symbiotic chimeras, was fiercely defended by Lynn Margulis. She than postulated that cells with nuclei (eukaryotic), emerged from bacteria through the Serial Endosymbiosis Theory (SET) that included: (I) that motility being acquired through anaerobic symbiosis between spirochaetes and other bacteria such as *Thermoplasma* (6); (II) postulated that mitochondria—found in all eukaryotic cells—evolved from Rickettsiaceae-like aerobic members of the Alphaproteobacteria and (III) that chloroplasts evolved from endosymbiotic Cyanobacteria. Recently, with the wealth of microbial community surveys and single cell sequencing, new lineages were revealed, including the non-extremophilic archaea 'DPANN' (Diapherotrites, Aenigmarchaeota and Nanoarchaeota) and the TACK superphylum group (Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota), both close to the Crenarchaeotes and linked to Eukaryotes (7). Genomic sequencing revealed a new view of the tree of life, which confirms that the eukaryotes branch within the TACK superphylum (8). Fossil record indicates that eukaryotic proto-multicellular organisms appeared ~1.8 M years ago as the dating of *Grypania spiralis* (9), yet the first metazoan fossil is about 600 mya (*Doushantuo* embryos) (10), and the Amniota must have separated from their closest living

relatives, the Amphibia, at least 360 mya (11). Back when dinosaurs perished, the surviving mammals diversified into the newly available niches with the oldest placental mammal being about 160 mya (12,13). Paleontologists believe that humans evolved from *Gorilla-Pan*-human clade ~10 mya (14) while *Homo sapiens* evolved 300,000-year-old (15).

Dinosaurs, pangids, modern humans all these animals have evolved in the presence of microbial communities that have shaped their fitness and phenotypes. Indeed, all macroorganisms (hosts) have co-evolved with bacteria, archaea, and viruses (symbionts). In this sense, the microbiota refers to all of the microbial cells associated with an animal or plant (taxonomic composition), while the microbiome is the genetic information of the microbiota, its genes, and genomes. The metagenome, a term first coined by Handelsman (16) refers to the study of the collective genomes of a given sample (habitat). The "holobiont", as firstly proposed by Lyn Margullis, consists therefore of a host and its symbionts, and now the term "hologenome" describes the sum of the genetic information of both the host and its symbiotic microorganisms (17). The holobiont is, therefore, a complex unit, and the microbiome can be transmitted between generations via matrilineal lineage (18). Microbe-host interactions affect the fitness of the holobiont beyond immunology or physiology, including development, behavior, and predisposition to disease. The holobiont is, in fact, an essential unit of selection in biological evolution (19). As the evolution of life occurred towards more complex forms, organisms recapitulate the tree of life, wild hominids or humans, carry their own species cells, plus those of bacteria, archaea, protozoa and fungi, being the microbiota congruent with the relationships of the hosts (18).

How humans acquire their microbiota

Mammals evolved by acquiring a maternal microbiome from the birth canal, by which the first microbiota prime the immune system and the whole system physiology. Seminal work by Dominguez-bello showed that if a baby is born vaginally, acquires bacteria from the mother's vagina (mostly Lactobacilli), while if a baby is born via C-section, the bacteria acquired by the baby are mainly from skin (20). As these microbiota changes have enormous immunological implications to human development and predisposition to disease, new strategies are being developed to restore the stability of vaginally-born microbiota.

The complexity of the microbiota continues at the postnatal stage with feeding habits. Lactobacilli acquired during labor and at the mother's breast, can degrade lactose and use challenging to digest substrates such as milk glycans (21). These, in turn, are a feast for *Bifidobacterium* influential in shaping the immune system. Formula feeding, in turn, is known to alter the baby microbiota (22) and the modern practices of extracting milk, freeze-thawing in plastic devices adds certainly other effects that have been so far overlooked in microbiome and functional studies. The introduction of solids later, around 6 months, introduces new bacterial diversity that steadily increases until about 3 years old (23). Our gut harbors an array of different microbial populations, over 100 trillion microbial cells, together in close contact with the host response and influencing physiology, metabolism, nutrition, and immunity. Lumen particle-associated microbiota and free-living bacteria play important roles in the digestion of residual substrates after the absorption sites. While food lasts hours in the small intestine, in the large intestine transit time is longer (days); undigested proteins can be digested into amino acids, short-chain fatty acids and organic acids which in turn feed the mucosa and peripheral tissues (24). The large intestine has, therefore, more microbes, is less permeable, and has higher short-chain fatty acid content (25). The human-associated microbiota colonizes either the surfaces of the host epithelium or their fluids, including the skin (26), the airways (27), oral sites (28), vaginal/cervical (29) or the gut (30).

Interestingly, the main problem when doing microbial ecology studies of the gut, is to define functional redundancy of the microbial niches. A recent research selecting improved culture conditions and new protocols, identified many new bacterial species in the gut repertoire, illuminating the gut microbial dark matter (31). Humans depend on their commensal bacteria for development, nutrition, immunity, and overall contributions to the maintenance of health. Human health depends on the careful balance of the microbiome that if disrupted, enters dysbiosis (imbalance); and this has been shown to contribute to many pathologies.

Symbionts within and on us

We are all different; we all have different gene variants, metagenomes, and microbiomes. Work from Jill Banfield's lab (UC Berkeley) shown that each baby has a unique gut bacterial repertoire, even co-hospitalized premature infants rarely share microbes, indicating that inter-individual differences in the gut microbiota arise after birth (32).

Decades of research have revealed the composition of non-diseased microbial niches, which is normally described in Microbiology textbooks as "normal" microbiota. To consider any microbial composition as "normal" is complex. There is functional redundancy in the microbiota with different species using the same niches. We do not know what constitutes a healthy microbiota. Despite the fact that our basic knowledge of the "normal" microbiota is very scarce, we know that the oral cavity is composed mostly of bacteria such as *Streptococcus*, *Staphylococcus*, *Actinomyces*, *Veillonella*, *Fusobacterium or Porphyromonas* (33). The eye microbiota is composed of *Acinetobacter*, *Aeribacillus* or *Pseudomonas* (34), while the skin is mostly composed by Corynebacteriaceae, Propionibacteriaceae, and Staphylococcaceae *with an abundance of Propionibacterium* spp. in areas of sebaceous gland accumulation (35). The organ with the highest bacterial diversity is the gut, mostly composed by members of phyla Firmicutes and Bacteroidetes, dominant taxa includes *Bacteroides*, *Bifidobacterium*, *Prevotella*, *Ruminococcus*, *Streptococcus*, *Enterobacteriaceae*, *Lactobacillus*, *Akkermansia* among others (24). The simplest of the bacterial organs is the genital tract of women. The biochemical barrier of the vagina is a glycogen-rich environment that selects for *Lactobacillus* that breaks down the epithelial glycogen into glucose. Glucose is in turn metabolized into lactic acid, which helps maintain low pH (~4.0) and dominance of lactobacilli and related bacteria (36). Bacterial barcode sequencing revealed that the vaginal microbiota changes according to ethnicity, and may not always be dominated by Lactobacilli. Indeed Caucasians and Asians have higher amounts of vaginal Lactobacilli compared to Hispanics or Blacks (37).

Microbiome and diseases

Microbial links with cancer started decades ago when *Helicobacter pylori* were found to be involved in gastric carcinogenesis. The International Agency for Research on Cancer (IARC) back in 1994 identified *H. pylori* as one of the most important carcinogens (group1) (38) as it causes a mixed acute and chronic inflammatory reaction, and Cag-seropositivity is tightly associated with a higher risk for developing gastric cancer. Given that a relation between the microbiota, the metabolism of the body and the immune system has been well established in the advent of new sequencing technologies, other microbial "actors" are known to be over-represented in cancer. For instance, *Fusobacterium nucleatum*, an opportunistic commensal anaerobe of the oral microbiota, is dominant in the colon of patients with colorectal cancer (39). Interestingly, the same bacterium is also dominant in patients with oropharyngeal cancer (40). *F. nucleatum* is known to produce the virulence factor FadA—leads to an increase of colonic epithelial cell permeability (41). Fusobacterium-positive colon cancers, when treated with antibiotics, result in a reduction of the tumor growth, bacterial load and cancer cell proliferation, thus its prominent association with cancer progression (42). Other bacteria such as *Enterococcus faecalis* associated with gastric cancer, produces reactive oxygen species, and *Bacteroides fragilis*, activates the oncogene c-MYC that leads to the expression of inflammatory cytokines and colon carcinogenesis (43). The precise biological mechanisms by which the microbiota influences micro epithelial systems inducing carcinogenesis remains to be clearly understood. Bacterial toxins can directly damage host DNA via the production of reactive oxygen species enabling mutations (44) binding to E-cadherin on colonic epithelial cells, activating b-catenin signaling and resulting in dysregulated cell growth (45), or even through other proinflammatory pathways involving mucosal barrier breach mediated by NF-kB, fuels carcinogenesis (46).

The gut microbiome has also been recently implicated in the success of cancer immunotherapy. Experiments using gnotobiotic mice models showed that certain bacteria enhanced anti-tumor therapies, as the efficacy of PD-L1 blockage therapy suggesting the gut microbiome may influence the final clinical outcome. Higher gut microbiome diversity and the dominance of Ruminococcaceae and *Bifidobacterium longum* were found in responders versus non-responders, in melanoma patients enrolled in anti-PD-1 therapy (47). Besides, germ-free mice and antibiotic-treated mice models have shown an impaired efficacy of anti-CTLA-4 antibody treatment (48), which proves a direct role of the gut microbiota.

Bacteria have also been implicated in a variety of other cancers, namely in women, as microbes are capable of producing estrogen-metabolizing enzymes and therefore modulate estrogen serum levels. The microbiota, endogenous hormonal levels, and compounds that mimic estrogen, may likely increase the risk of developing hormone-related diseases. Recently, it was suggested that the microbiota of women with breast cancer differs in composition from that of healthy women (49), once again showing that bacteria may be associated with cancer development and with different responses to therapy. Breast cancer is directly related to higher levels of endogenous estrogens, especially in a post-

menopause stage, however, few studies have linked the microbiome with breast cancer. Nonetheless, the collection of bacteria capable of metabolizing estrogen and its metabolites, "estrobolome", is known to change the levels of circulating estrogens increasing or diminishing the risk of breast cancer. Regarding cervical cancer, information relating the microbiota and the mycobiome (bacteria and fungi respectively) is revealing complex inter-kingdom interactions regarding dysplasia and even HPV infections (29,40,50). Recent evidence shows that the cervicovaginal microbiota plays a role in the persistence of the virus and subsequently of cervical disease. *Lactobacillus spp*. is protective against acquisition and persistence of HPV and ultimately, development and progression of CIN (51). Lactobacilli express enzymes capable of glycogen fermentation and the drop in pH in the cervicovaginal environment thus inhibiting the growth of potentially pathogenic species, working as a protective barrier against infections. Changes in these lactic acids will likely lead to altered microbial equilibrium and thus cervicovaginal integrity. Species such as *Lactobacillus crispatus* were described to be able to excrete *Lactobacillus* epithelium adhesin (LEA), mediating adhesion to the genital mucosa while contributing to inhibiting the adhesion of *Gardnerella vaginalis* (52). Bacterial signatures typically associated to diseases such as Bacterial Vaginosis (BV) or HPV infections include *Gardnerella vaginallis*, *Sneathia* or even *Prevotella*, *all* anaerobes that typically appear when the mucosa is devoid of *Lactobacillus* (53). A pioneer study in Hispanics revealed that lower amounts of Lactobacilli and increased abundance of *Atopobium vaginae* and *Gardnerella vaginalis* are related to high-risk HPV infections and with cervical intraepithelial neoplasia, as were increased numbers of *Malassezia* yeast (29). These yeast are a part of the resident genital microbiota in healthy men and have been found to participate in cutaneous carcinogenesis (54). This finding showed how neglected are fungi in microbiome studies and highlighted the need for understanding the influence of fungi in bacterial dynamics. The mycobiome (fungal diversity) has been poorly studied and may contribute to essential functions, including bacterial community assembly and resistance to infections.

Microbes also influence drug metabolism and bioavailability (e.g., inactivating substrates through promiscuity and competence). Recent studies have shown the active role of the gut microbiota on drug and xenobiotic metabolism with direct consequences for both efficacy and toxicity. Indeed, it is known that bacteria such as gut *E. lenta* are capable of inactivating a cardiac drug, digoxin—a natural supplement used to treat chronic heart failure and atrial fibrillation (55). The activity of nitroreductases by the gut microbiota (nitro-groups are reduced to amines), was found to be important in drugs with these functional groups such as the anti-anxiety agent bromazepam (56). Oral administration of probiotics was also shown to selectively change the gut microbiota composition in mice and alter drug toxicity. A pharmacokinetic study with orally-administered acetaminophen jointly with different probiotics showed differentially increased degradation of acetaminophen by gut microbiota. This study demonstrated that the intake of probiotics leads to variable absorption of this orally administered drug, through differentially gut microbiota-mediated drug metabolism, that could result in altered systemic concentrations of the drug (57). Also, regarding hepatotoxicity induced by Acetaminophen, which is one of the leading causes of acute liver failure worldwide (58), a study shown that the gut microbiota is a known modulator of the diurnal variation of its hepatotoxicity (59). There are many ongoing studies on microbiome-mediated pharmacokinetics, which should reveal new and exciting relationships of keystone taxa with drug bioavailability and toxicity. Clinical guidelines should include the presence, abundance, and expression levels of microbial genes known to play important roles in the metabolism of a given drug.

Microbiome implications to disparities research

To be able to reduce disease burden, it requires a thorough understanding of all the determinants that together influence the development of a certain disease. These multiple factors include the immune system, lifestyle, genetics, diet, which have all been implicated as causing changes to the microbiome. The fact that there are ethnical changes associated to the microbiota, suggests that these may contribute to racial and ethnic health disparities—defined as avoidable health differences in the incidence, prevalence, mortality, and burden of among specific population groups in the US (60).

Factors such as diet and lifestyle behaviors are known to influence ethnic differences in health, and as these also influence the microbiome, in which its composition may result in a given health outcome. The previous example of the vaginal microbiota having less of dominance in Lactobacilli—"the good guys"—in Latinos and Blacks, may leave these groups more susceptible to diseases such as transmitted

sexual infections or even anogenital cancers. Habits shared by African Americans and also Latinos, such as vaginal douching/cleansing with a liquid solution, are associated with bacterial vaginosis, cervical cancer and preterm birth (61). This habit leads to changes in the vaginal microbiota, therefore, causing a state of dysbiosis, which can lead to proneness to the development of several different infections.

Studies of the gut microbiota revealed differences between European and rural African children (62) and differences were also found between the dietary content depending on fiber and fat, heavily influenced by geography between either Africans and African Americans (63). Rural peoples have higher bacterial diversity, including those with hunter-gatherer lifestyles (63). In disparities, psychosocial and food-related environmental risk factors are linked to cardiometabolic conditions (obesity and diabetes) and these, in turn, may have a microbiome-based origin, and may require additional strategies to improve microbiome health.

Lifestyle activities that lead to dysbiosis include the use of antibiotics, delivery mode, diet, sleep disruption or the fact that we spend 90% of our time indoors in contaminated non-oxygenated built environments, it all contributes to changes in human microbiome including emotional health outcomes. Additionally, other lifestyle impacts such as exposure to chemicals from food preservatives, agriculture, and industrial production may impact the gut microbiome by disrupting microbial biosynthetic pathways which in turn can lead to gut signaling dysfunctions that have linked chemicals and neural pathophysiological problems (64). The diseased phenotypes due to microbial disruptions include obesity, ulcerative colitis and Chron's, asthma, behavior, or autism. Therefore, it is essential to include microbiome approaches in health disparity studies, individuals from diverse ethnic, cultural, and social backgrounds. This will be key to establish links between specific disease phenotypes in different ethnic groups, shedding light on why some groups have higher prevalence, risk, or severity of certain diseases. This will also define future use of joint therapies and treatments, including those that help improve microbiota dynamics and guarantee a microbiota that may improve treatment success. Therapies being now used to overcome these gut problems, mostly *Clostridium difficile* infections, include Fecal microbiota transplantation (FMT), where fecal matter with beneficial microbes are transferred from a healthy donor to the diseased recipient.

FMT success is based primarily on a positive clinical response in the recipient and shift towards healthier microbiomes, resistant to infections. It is imperative to select a good donor and now new approaches are being done to include a universal donor, using keystone species as predictors of FMT success (65).

Considerations for microbiome studies

We are living in a microbiome era. In the last ten years, and especially since the 2016 White House announcement of the National Microbiome Initiative, numerous grants value the inclusion of aims with microbiome research. This indeed advances microbiome science in ways that will benefit not only individuals, their communities but also the planet. However, there are still many problems in the establishment of these projects that include poor experimental design, limitations with patient recruitment, limited knowledge on sample handling, storage, DNA extraction and especially quality control on sequence data and microbiome analyses.

When starting a protocol for patient recruitment aimed at analyzing the microbiome associated to any body site, it is essential to consider the intake of antibiotics (at least had not taken antibiotics in the last month). Currently, there are efforts to standardize genomic DNA extractions (66) and analytical protocols (67) and to increase the availability of the sequencing data. Advances in next-generation sequencing methods, like pyrosequencing (68) and more recently Illumina sequencing (69) of 16S rDNA genes, exponentially increased the number of studies and their relationships with disease phenotypes (8,70-74). Studies vary on the 16S variable region, with the preferred 16S rDNA V4 been shown to outperform that of V6 and being the most widely used for microbiome studies across the board (75). The V4 region is recognized as the most reliable sub-region for representing full-length 16S rRNA sequences with a superior phylogenetic resolution (76). Poor quality reads, unassigned taxa, reads homologous to chloroplasts and mitochondria should be removed from the datasets, to avoid inflating alpha diversity estimates. Databases for taxonomy assignment include the Greengenes-based classifier that has been trained on the 515F/806R region of the 16S (77,78); Silva (79) (ARB compatible) (79) the RDP database (80) and UNITE for fungi (81). The new QIITA pipeline (https://qiita.ucsd.edu) has revolutionized data analyses for biologists including an ease to share data among collaborators and also to submit to reads to public databases, as EBI submission is facilitated via this platform (67). QIIME2 version of the QIIME pipeline (qiime2.org) implements analyses of exact sequence variants

ASVs, through algorithms implemented via plug-ins that allow for taxonomic classification using DADA2 and Deblur. The established 97% similarity between sequences has been shown to represent different species possibly. Most of these sequence variants in QIIME2 have species-level assignments that can be given by any of the supported databases such as Greengenes, Silva or others.

Shotgun metagenomics is another widely available tool for the study of microbiomes with an additional resolution to account for function. It corresponds to the sequencing of all the genomes of all organisms present in a sample, offering an unprecedented opportunity to acquire the gene catalogs of microbes providing a new vision on the functional possibilities of the microbes (16,82). This technique offers a resolution of the genomic composition of the communities and includes read preprocessing (QC), assembly and gene prediction and annotation. Single cell sequencing is another powerful tool for characterizing rare cells/populations (83). Recently, many new lineages of the tree of life have been discovered and characterized phylogenetically due to single cell sequencing, unravelling the microbial dark matter. The methods involved in the production of this type of data include high-throughput single-cell flow sorting, whole-genome amplification, and SSU rRNA screening of single amplified genomes.

Other approaches that complement the microbiome include the characterization of the metabolic pathways, fluxes, and metabolite biomarkers through metabolomics. Mass spectrometry, in combination with chromatography or nuclear magnetic resonance, is used to resolve metabolic patterns and reveal the diversity of molecules available in a given habitat.

Nowadays new efforts have been done to cultivate those bacteria who were previously unculturable (84), which opens the possibility of characterizing phenotypically and biochemically bacteria that were only available through sequencing. An approach that could reveal details for example on oxygen-sensitivity of intestinal bacteria, sporulation capacity and its implications to the study of gut microbial ecology and the new therapies such as fecal matter transplants (85).

Conclusions

The microbiome is now regarded as a crucial component of human health, moderating all interactions between the environment and the host's immune response, between diet, drugs, supplements and the hosts' metabolism. Omics technologies are now regarded an essential component in complementing clinical research, helping not only to define the keystone members of the community in health and disease but also defining their functional profiles and their interactions with the hosts' genome. In the future, personalized medicine will use tailor-made, microbiome-based diagnostics and treatments, and prevention approaches will include a detailed profiling of the microbiome of individual patients. Identifying certain "keystone species" will help identify targets for manipulating the microbiota to improve the health of patients. Schools, therefore, need to update their curricula to include bioinformatic analyses tailored to metagenomic studies, and Medical Schools need to implement courses where MDs can reflect and appreciate human microbiome in health and disease to better come up with treatments and therapies.

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Footnote

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