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Review

The human microbiota and infection prevention

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Abstract

The human microbiome participates in numerous aspects of human physiology and disease states. Recently, studies have begun to explore the role of the microbiome in colonization, infection and transmission of pathogens. This review provides a summary of the methodological principles used in microbiome studies and the published evidence of the impact of microbiome dysbiosis in infection prevention.

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The human microbiome consists of the total microbial community (or microbiota) and the associated biomolecules. It is composed of 10–100 trillion microbial cells (10× more than human cells containing genetic material), and it contains >1,000 bacterial species and 100-fold more genes than the human genome.[1–3] A study in the early 2000s by Eckburg et al[4] analyzed the microbial composition of human fecal samples using 16S rRNA gene sequencing. They showed that 60% of the identified bacteria corresponded to novel organisms and 80% of sequences belonged to previously uncultivated bacterial species.[4] These striking findings, of a previously underrecognized and immense diversity of the intestinal microflora, have led to a tremendous focus on the microbiota. Culture-independent techniques, which identify microorganisms based on DNA sequences directly from the sample, have begun to elucidate the complex composition of microbial communities. Functional metagenomic and metabolomic techniques have also begun to describe the biological tasks of the microbiome.

The microbiome participates in numerous aspects of human physiology including the development of the immune system, energy metabolism, and intestinal endocrine functions.[5,6] It performs other essential functions such as the production of vitamin B and K groups and the degradation of complex carbohydrates from ingested plant-derived fibers.[7] Imbalances in the human microbiome, often referred as dysbiosis, induced by lifestyle factors, diet, and antimicrobials, have been implicated in obesity, cardiovascular and autoimmune diseases, malignancies, and infections.[5,8] Given its potential role in disease states, interventions to restore the microbiome, such as fecal transplantation and the development of consortia of beneficial bacteria, are under investigation as potential therapeutic options.[6]

The objective of this paper is to provide an overview of the current approaches for assessing the microbiome and the implications that altered, or dysbiotic, microbiomes may have in infection prevention. The review also focuses on methodological principles to promote understanding of the complexities of microbiome research to facilitate more complete interpretation of the literature. Common terms used in microbiome studies are shown in Table 1.

Composition of the microbiome

The tree of life consists of 3 domains: Archea, Eukarya, and Bacteria. The Bacteria domain includes 29 phyla, of which 6 predominate in the human microbiome: Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Cyanobacteria, and Fusobacteria.[9] The type of bacteria within each of these phyla are shown in Table 2. The relative abundance of the members of these phyla varies among different sites in the body (Figure 1).[10] In the healthy gut, Firmicutes and Bacteroidetes represent >90% of the bacterial community. At lower phylogenetic levels, such as the genus or species level, the gut microbiota is vastly diverse among individuals. This high interindividual variability disproved the initial hypothesis that postulated the existence of a taxonomical core shared by most individuals and has posed a significant challenge for defining what we understand to be a healthy human microbiome. Although the human microbiome varies over time within individuals, the extent of its longitudinal variation is significantly lower than the variability observed between hosts, indicating that the human microbiome is individualized.[2] In contrast to the taxonomical variability observed between subjects, the functional metagenomic prediction of the metabolic pathways present in the human microbiome showed that most individuals share the same gene-associated functions, suggesting the existence of a functional core among the human microbiomes.[11] The microbiome individuality and functional stability are thought to be key features of the healthy human microbiome and are the focus of intense investigation.[3,4]

One of the approaches most commonly used to study the microbiome is based on the amplification and sequencing of the 16S rRNA gene of Archaea and Bacteria. The 16S gene has regions that are highly conserved adjacent to regions that are highly variable among prokaryotes. These characteristics make the 16S gene an ideal marker for cataloging microorganisms. First, based on its conserved regions, it is possible to use universal primers to detect and amplify bacterial DNA from almost any sample. Second, by sequencing its variable regions, it is possible to group the sequences obtained into molecular

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Table 1. Common Terms in Microbiota or With Particular Relevance to Infection Prevention

Microbiota – the microbial community in a specified environment.
Microbiome – the total DNA of all microorganisms, including bacteria, fungi, viruses, protozoa and archaea within a human or animal host.
Taxa – a group of related organisms that are classified together at the same phylogenetic level (eg, phyla, family, genus, species).
Dysbiosis – imbalances in the microbiota.
Colonization resistance – the effect of the microbiome in protecting the host from becoming colonized with pathogens, mediated through direct or indirect functions of the microbiome.
Resistome – the pool of antimicrobial resistance genes within the microbiome.
Metabolome – the set of chemicals produced by the microbiota.
Alpha diversity – within-sample diversity, total number of taxa or evenness.
Beta diversity – between-sample diversity, differences between two samples, such as sites, disease-states or patient populations.
Protective taxa – a specific taxa that appears in cross-sectional, or ideally, natural history studies, associated with a decreased risk of pathogen, such as an MDRO or *C. difficile*, colonization or infection.

Note. MDRO, multidrug-resistant organism.

Table 2. Composition of Phyla Present in the Human Microbiota

Phylum	Predominant Type of Bacteria	Genus Examples
Bacteroidetes	Anaerobic and aerobic gram-negative rods	*Bacteroides* spp
Firmicutes	Gram-positive bacteria with low G+C content	*Clostridium* spp *Lactobacillus* spp *Staphylococcus* spp
Actinobacteria	Gram-positive bacteria with high G+C content	Corynebacterium spp *Cutibacterium* spp
Proteobacteria	Gram-negative bacteria	*Escherichia* spp *Acinetobacter* spp *Pseudomonas* spp
Fusobacteria	Anaerobic gram-negative rods	*Fusobacterium* spp
Cyanobacteria	Blue-green algae	

operational taxonomic units (OTU) based on a predefined sequence similarity threshold, usually 97%. Thus, these OTUs refer to clusters of organisms, grouped by DNA similarity, and they are usually taken as a species surrogate for diversity analyses.[12]

Some limitations of the 16S targeted approach have been described. First, the 16S gene is subject to variation in the number of copies per cell, which may affect accuracy, particularly when estimating microbial abundances. Second, the amplification step is susceptible to biases introduced by the propensity of primers to hybridize more efficiently and of amplification to proceed for some bacterial 16S sequences over others, which may also lead to misrepresentation of the relative abundances of community members. Third, the 16S gene does not provide information regarding whole genomes; therefore, inferring functional roles from the microbial community, although possible, is limited. Finally, 16S gene sequencing approaches only identify Bacteria and Archaea, leaving other microorganisms that are part of the human microbiome, such as viruses and eukaryotes, out of the analysis.[13,14]

Whole-metagenome shotgun sequencing

In addition to 16S rRNA sequencing, another approach to study microbial communities is whole-metagenome shotgun (WMS) sequencing. This term refers to the untargeted process of sequencing the entire pool of DNA extracted directly from a sample

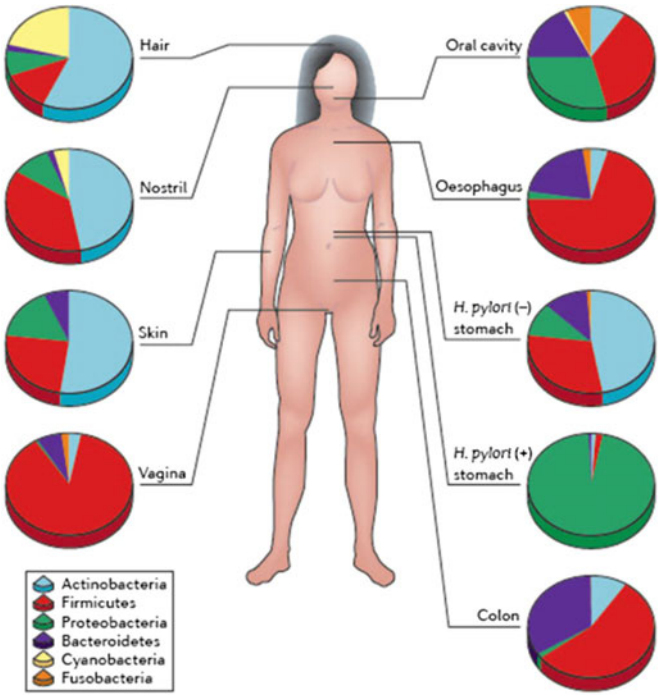

Fig. 1. Compositional differences in the microbiome by anatomic site.[10]

(the mixture of genomes, or metagenome).[15] Concerning taxonomic profiling studies, WMS sequencing is not subject to PCR-related biases, it is not affected by the variable copy number of the 16S gene, and compared to the 16S targeted approach, it provides higher biological resolution even at the species and strain level. Additionally, WMS sequencing can provide meaningful data about the functional potential of microbial communities, such as antimicrobial resistance genes and biochemical compounds they produce.[16,17] Higher costs compared to the 16S approach, and significant computational and analytical challenges, however, are still major limitations of this sequencing method. Current efforts to integrate WMS sequencing with other techniques, including metatranscriptomics (the activity of present genes) and metabolomics (the metabolic products), will be key for linking metagenomic data with the terminal bioactive products of the microbial community.[13]

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Biodiversity of the microbiota

One of the main goals of microbial community analyses is to determine not only its composition but its community structure, or diversity. Two important parameters are commonly used for describing microbial diversity: alpha diversity, or within-sample diversity, and beta diversity, or between sample diversity. Alpha diversity can be described regarding its richness (ie, the total number of taxa observed in a sample), its evenness (ie, how balanced are the relative abundances of the community members), and its phylogenetic relationships. These characteristics are complementary and show different aspects of the community assembly. Commonly used alpha diversity measures include directly counting the number of taxa present in a sample (richness), and the use of parameters that consider both richness and the distribution of the relative abundances of community members, such as the Shannon and inverse Simpson indices. These metrics are accurate at estimating microbial diversity based on the most abundant taxa, but their performance decreases when addressing the contribution of the less abundant members of a community. Despite its inherent limitations, alpha diversity estimation is useful for quantifying changes in microbial diversity associated with the different situation of interest, such as antimicrobial exposure, or a particular disease.[12]

In addition to alpha diversity, microbiome analyses also examine the "between sample" diversity or beta diversity. Beta diversity estimates the degree of similarity or difference in the taxonomical composition between samples or group of samples. Beta diversity metrics are diverse and inform different aspects of community composition when comparing samples. Qualitative measures consider the presence or absence of features, and quantitative estimators consider the relative abundance of community members. The third class of beta diversity measures includes phylogenetic information coupled either with qualitative or quantitative data. Beta diversity data are frequently summarized using ordination techniques, such as principal coordinates analysis for visualizing and exploring sample clustering according to metadata of interest. Samples that cluster together are more similar than samples that cluster apart.[12]

A key concept that affects both 16S rRNA amplicon sequencing and WMS sequencing is the sequencing depth, which refers to the total number of DNA sequences per sample obtained after completing the sequencing process. Similar to the sampling effort in ecology, the sequencing depth significantly impacts the biodiversity observed in a sample; more deep sequencing efforts have a higher probability for detecting the less abundant members of a community. In other words, "the more you sample, the more you find." Microbiome studies usually report a sample's sequencing depths using summary statistics and rarefaction curves. The latter put summary statistics into context by plotting curves that show the association between the number of sequences retrieved from each sample and the expected diversity of the sample based on the observed abundances. Diversity analysis should be conducted at a sequencing depth approaching the saturation point for new species discovery to provide meaningful data. Furthermore, samples usually yield variable number of reads, posing a challenge to differentiate between true biologic variation versus dissimilar sequencing efficiency. Different analytic approaches (ie, normalization methods) are commonly used to account for variable library sizes before comparing diversity metrics between samples or groups of samples.[18]

Microbiome dysbiosis and its impact on colonization and infections caused by multidrug-resistant organisms and other pathogens

Age, diet, and geographical distribution are important factors that shape the microbiome and explain in part its compositional variability.[10,19] Similarly, exposure to some drugs has been associated with significant changes in the structure and composition of the human microbiome. Antimicrobial exposure profoundly affects the microbiome structure, leading to a decrease in bacterial diversity and to both decreases and blooms of specific taxa.[20,21] The effects can be long-lasting. Dethlefsen et al[22] showed that a 5-day course of ciprofloxacin could cause microbiome dysbiosis for up to 5 months. Substantial changes in microbiome composition for up to 4 years have also been found after a 7-day course of clarithromycin, metronidazole, and omeprazole.[23]

The key concept pertaining to antimicrobial exposure and the microbiome is a decrease in "colonization resistance." This term refers to protective taxa within the microbiome that reduce the risk colonization with a pathogen, mediated either through functions that directly inhibit growth of the organism, for example, competition for nutrients or the direct expression of inhibitory or toxic substances, or through functions that interact with the host to indirectly inhibit growth of a pathogen, for example, stimulation of the host's innate immunity.

Several protective taxa have been identified. Caballero et al showed that *Blautia producta* restores colonization resistance against vancomycin-resistant *Enterococcus* (VRE) and directly inhibits VRE growth in murine models.[24] In vitro studies have shown that the commensal bacterium *C. scindens* converts primary bile acids to secondary ones, and mathematical models have shown that the absence of *C. scindens* in the gut promotes *C. difficile* infections, since secondary bile acids inhibit the germination of *C. difficile* spores.[25] Negative and positive correlations with *C. difficile* infections have also been shown with other taxa.[26,27] Thus, reconstitution to a healthy microbiome via fecal transplant has been associated with preventing *C. difficile* infections and is now recommended as a treatment option for patients with multiple recurrences.[28] *Lactobacillus* spp have also been implicated in colonization resistance. Comparison of the fecal microbiome among hospitalized patients exposed to antimicrobials, who acquired or did not acquire a multidrug-resistant organism (an MDRO), identified a greater abundance of *Lactobacillus* spp among those who did not acquire an MDRO, suggesting that these bacteria may have a protective role against MDRO colonization.[29]

Domination of a particular taxa can also lead to an increased risk of infection. Using 16S rRNA gene sequencing, Taur et al[30] showed that intestinal domination (>30% of the microbiota) by *Enterococcus* spp and Proteobacteria (a phylum of gram-negative bacteria) increased the risk of VRE bacteremia by 9-fold and gram-negative rod bacteremia by 5-fold. In a study of long-term acute-care residents, an increased relative abundance of carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) in the gut was associated with an increased risk of KPC-Kp bacteremia.[31] Although likely correlated, further studies are needed to determine whether the ratio of the dominant taxa to other taxa in the microbiome or the actual bacterial load of the dominant taxa affects the risk of subsequent infection.

Antimicrobials also affect the gut resistome, defined as the pool of antimicrobial resistance genes within the microbiome. Increases in the abundance of antimicrobial resistance genes occur with

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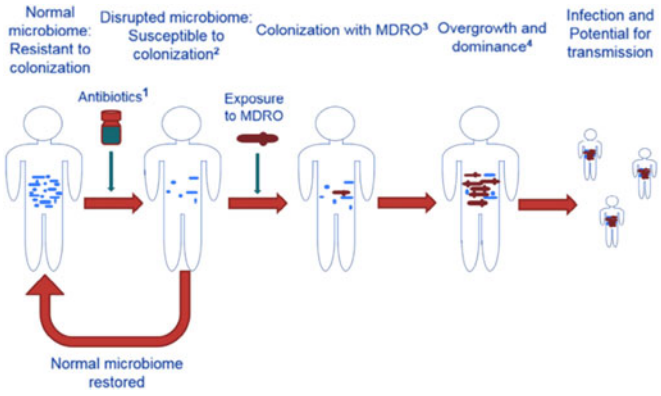

Fig. 2. Causal pathway from health to disease: microbiome disruption indices (MDI). [1]Antibiotic MDI indicates the potential an antibiotic has for disrupting the intestinal microbiome. [2]Disrupted microbiome status MDI characterizes the degree and type of disruption in the intestinal microbiome and the susceptibility to colonization by a MDRO. [3]MDRO colonization MDI indicates susceptibility to overgrowth and dominance by a MDRO. [4]MDI characterizing overgrowth and dominance by a MDRO indicates susceptibility for infection with a MDRO and the potential for transmission to others through skin-environment contamination.[39] Note. MDRO, multidrug-resistant organism.

antimicrobial exposure.[32] In a study of recurrent *C. difficile* infection among patients with repeated antimicrobial exposure, the abundance of β-lactam, fluoroquinolone and multidrug efflux-pump–resistant genes was higher than in healthy controls. Moreover, fecal microbiota transplantation reduced the load of these genes.[33] Metagenomic analyses among antimicrobial exposed patients who acquired an MDRO also revealed a higher abundance of genes related to several pathways implicated in multidrug resistance, including the 2-component system, the ATP-binding cassette system, and the phosphotransferase system.[34]

Importantly, nonantimicrobial medications also lead to microbiome dysbioisis and an increased risk of colonization with pathogens. A systematic review of medications associated with gut dysbiosis identified proton pump inhibitors, metformin, and nonsteroidal anti-inflammatory agents with changes in the structure of the microbial gut composition.[35] Nonantimicrobial medications have also been associated with an increased risk of MDRO acquisition. In a nested case-control study of 137 nursing home residents who were not exposed to antimicrobials, of whom 32% acquired an MDRO, exposure to laxatives and acid reducers was significantly associated with a greater risk of acquisition compared to those who did not receive these medications.[36]

Infection prevention strategies

Although antimicrobial stewardship and prevention of transmission through hand hygiene and contact precautions has decreased the spread of MDROs, the problem persists. Innovative strategies are needed. An intact microbiome is a host defense mechanism for preventing colonization and infection with MDRO and other pathogens. Dysbiosis leads to colonization and dominance of MDROs and other pathogens and is a risk factor for infection. Dominance of a particular taxa in the gut has also been associated with greater environmental contamination, which implies a greater risk of transmission.[37] Recently, the Centers for Disease Control and Prevention has begun to study "microbiome disruption indices" (MDI), characteristics of the microbiome structure and composition, its resistome, and the biochemicals it produces (metabolome), that can identify patients at high risk of colonization with, infection with, or transmission of MDROs and other pathogens (Figure 2).[20,38] Studies have begun to characterize these MDIs, as mentioned above. Several key questions require further study: (1) What are the MDIs that promote colonization and infection with pathogens? (2) What are the cumulative MDIs that increase the risk of transmission? (3) What are the differences in MDIs induced by different antimicrobials? And (4) are there specific antimicrobials that have minimal effect on the microbiome or, of least duration?

Future directions

Despite the tremendous number of publications pertaining to the role of the microbiome in infections and other disease states in the last decade, and even journals dedicated only to microbiome research, the study of the microbiota, in the area of infection prevention, is still in its infancy. Considerable research is needed to meet Koch's postulates for establishing a causative relationship between specific characteristic of the microbiome bacterial compositions. Other research areas include the role of taxa that may not be detected due to very low bacterial loads or insufficient sequencing depth, the resistome and metabolome components of the microbiome, and the role of a dysbiotic microbiome in pathogen transmission.

Recent clinical trials are focusing on restoring the microbiome to prevent infections. A randomized double-blinded, placebo-controlled phase 2B trial of a microbiota-based drug, RBX2660, showed promising results in the prevention of recurrent *C. difficile* infection.[39] Looking forward, a similar "pill" could be developed to prevent colonization, infection or transmission of MDROs. The current research strongly suggests that this is a real possibility.

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