

# Human Gastrointestinal Microbiota

Subjects: Gastroenterology

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## Definition

The human gastrointestinal microbiota (GIM) is a complex and diverse ecosystem that consists of community of fungi, viruses, protists and majorly bacteria. The association of several human illnesses, such as inflammatory bowel disease, allergy, metabolic syndrome and cancers, have been linked directly or indirectly to compromise in the integrity of the GIM, for which some medical interventions have been proposed or attempted. This review highlights and gives update on various technologies, including microfluidics, high-through-put sequencing, metabolomics, metatranscriptomics and culture in GIM research and their applications in gastrointestinal microbiota therapy, with a view to raise interest in the evaluation, validation and eventual use of these technologies in diagnosis and the incorporation of therapies in routine clinical practice.

## 1. Introduction

The human gastrointestinal microbiota (GIM) is a complex and diverse ecosystem that consists of community of fungi, viruses, protists and majorly bacteria.

Prior to its birth, it is presumed that the unborn is free of microbial flora, and that at birth, the infant first comes in contact with the resident microbial flora of the mothers' vagina if birth was through the natural birth canal, or the microbial flora of the mothers' skin if birth was through cesarean section<sup>[1][2][3]</sup>. Although some studies<sup>[4][5][6]</sup> have suggested the early inoculation of the fetus with bacteria and bacteria DNA through the placenta. The study by de Goffau et al.<sup>[7]</sup> reported that the human placenta has no microbiome. Detected bacteria were acquired during labor and delivery. After birth, according to the findings of Koenig et al.<sup>[8]</sup>, there were apparent chaotic shifts of microbiome from that endowed with genes facilitating lactate utilization and plant polysaccharide metabolism mediated by milk-based diet to increase in *Bacteroidetes* initiated by introduction of solid food that prepares the infant gut for adult diet. However, in the findings of Differding et al.<sup>[9]</sup>, the early introduction of infants to complementary food was associated with altered gut microbiota composition and butyric acid concentration, which have been previously identified as precursors to oxidative stress, immune disorder and obesity in childhood.

The microbiome of the adult gut accommodates various communities of phylotypes belonging to the phyla *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Fusobacteria*, *Firmicutes* and *Verrucomicrobia*<sup>[10]</sup>. Most of these phyla are present in the stomach, small intestine and colon. However, the colon is more populated with several genera belonging to the afore mentioned phyla, including the genus *Akkemansia* that belongs to the phylum *Verrucomicrobia*, which has been found to be limited in patients with obesity, inflammatory bowel disease and other metabolic syndromes, while it is in abundance in the biopsies of healthy individuals<sup>[10][11]</sup>. As has been reported in several studies, dietary types and pattern shapes and determines the diversity of the gut microbiome. In the submission of Amabebe et al.<sup>[12]</sup>, high fat and carbohydrate diet builds a gut microbiota that is predominated by *Methanobrevibacter*, *Firmicutes* (*Clostridium*) and *Prevotella* and deficient in bacteria such as *Bacteroides*, *Lactobacillus*, *Akkemansia* and *Bifidobacterium*. Barone et al.<sup>[13]</sup>, in their study brought to the fore the impact of modern Paleolithic diet (MPD) that consist of vegetables, seeds, lean meat, fruits, eggs, nuts and fish on the gut microbiome. They observed that the gut microbiome of urban Italians adhering to MPD showed an ample degree of biodiversity with high relative abundance of fat-loving and bile tolerant microorganisms. As have been mentioned earlier, perturbations or dysbiosis in combination with altered permeability are crucial mechanisms that mediate disease manifestation<sup>[14]</sup>. Fecal microbiota transplantation (FMT) has gained relevance in recent times in the treatment and correction of gut infections or disorders that might have resulted from the depletion of resident microbiota and infection by pathogenic bacteria. Huge successes have been recorded in FMT therapy, with about 92% efficacy reported in the treatment of recurrent *Clostridium difficile* infection<sup>[15]</sup>. In a recent study by Zou et al.<sup>[16]</sup>, it was shown that patients with Crohn's disease and ulcerative colitis that had FMT were in remission after three days of transplant with notable bacterial colonization of the gut. FMT therapy has been extended to the treatment of lifestyle and other diseases,

such as diabetes, metabolic syndrome, Parkinson's disease, obesity and cancer. FMT entails transfer of gut microbiota in feces of a healthy donor to recipient patient to correct/treat a disorder or gastrointestinal disease<sup>[17][18][19]</sup>. Although the level of success of this procedure, is yet to be wide spread due to some constraints identified by Cammarota et al.<sup>[20]</sup>, including difficulties with donor recruitment, lack of dedicated centers and issues pertaining to safety monitoring and regulation, hence, the proposal for the provision of stool banks to bridge the gap of FMT in clinical practice.

The afore mentioned technique offers a natural option to routine medical treatments of chronic ailments by providing direct and effective remedy preventing dysbiosis in the host, thereby improving health conditions<sup>[21][22]</sup>.

## 2. Technologies in Gastrointestinal Microbiome Study

Since the structure, composition and diversity of the human gut microbiota has been correlated with the health status of humans, it could be presumed that the future of combating certain ailments is through exploring individualized gastrointestinal microbiome as the gastrointestinal microbiome era heralds. In the past, scientists have used culture independent techniques such as electrophoresis based methods, including denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and PCR based methods, such as terminal restriction fragment length polymorphism (T-RFLP) and random amplified polymorphic DNA (RAPD), to study the community structure, diversity and genetic relatedness of bacteria in communities. Fluorescence in situ hybridization (FISH) is a cytogenetic technique that has been used in the study of individual microbes within gut microbiota, such as *Listeria monocytogenes*, *Salmonella* species, *Helicobacter pylori* and *Yersinia enterocolitica*, which are gut pathogens<sup>[23][24][25][26]</sup>. Russmann et al.<sup>[27]</sup> used FISH in the diagnosis of *Helicobacter pylori* cultured isolates, and the same technique was used to proffer antibiotic treatment options. These methods had a lot of drawbacks, including the need for specific probes, low resolution, specificity and sensitivity. However, advances in sequencing and culture technologies have paved the way to analyzing big data arising from exploration of the rich microbiome ecosystem of the gut, which is evident in several studies, as shown in Table 1. Such technologies are high-throughput sequencing, microfluidics, high-throughput metabolomics, assays engineered organoids derived from human stem cells and high-throughput culturing<sup>[28]</sup>. They have far reaching advantages over the older or traditional technology already mentioned, but with some limitations as well (summary in Table 2). The pros and cons of these technologies are described below.

**Table 1.** Studies on Microbiome, Outcomes and Methods Employed.

Subject	Methods Employed	Outcome	Reference
Association between breast milk oligosaccharides and fecal microbiota in healthy breast fed infants	16S rRNA genes sequencing of V4 region using the Illumina Hiseq 2000 platform, porous graphitized carbon-ultra high-performance liquid chromatography (PGC-UPLC-MS) and bioinformatics (QIIME)	Microbiota composition strongly influenced by infant age, associated mode of delivery and breast milk	<sup>[29]</sup>
Dynamics and stabilization of the human gut microbiome during the first year of life	Metagenomics (DNA extraction from stool samples and preparation of DNA library using Illumina Hiseq2000) and bioinformatics (SOAPdenovo2, GeneMark v2.7, NCBI database)	Nutrition has a far reaching influence on infant microbiota composition and function with halting of breast-feeding other than introduction of solid food	<sup>[30]</sup>

Determining the diversity of human gut microbiota	Culture with enrichment, 16S rRNA gene sequencing of V3 region using the Illumina Miseq platform and bioinformatics (QIIME)	Use of enriched culture method enhanced the culturability of bacteria identified by 16S sequencing of the microbiota of the human gut	[31]
Impact of diet during pregnancy on maternal microbiota clusters and its influence on neonatal microbiota and infant growth during the first 18 months of life	16S rRNA gene sequencing of V3-V4 region using Miseq Illumina platform. Bioinformatics (QIIME, LEfSe, Calypso online platform)	Diet is an important perinatal factor in the initial phase of life and have significant impact on neonatal microbiome	[32]
Heritable components of the human fecal microbiome are associated with visceral fat	Measuring of body composition by dual-energy X-ray absorptiometry, 16S rRNA gene sequencing of V4 region on Illumina Miseq platform and bioinformatics (QIIME 1.7.0, PICRUST v1.0.0, STAMP)	There was significant association of adiposity-OTU abundance with host genetic variations indicating possible role of host genes in influencing the link between obesity and fecal microbiome	[33]
Succession of microbial consortia in the developing infant gut microbiome	454-pyrosequencing of 16S rRNA gene, GC-MS analysis of SCFA, quantitative PCR and bioinformatics (QIIME, MG-RAST, NCBI database)	Revealed shifts in microbiome associated with life events	[7]
Identification of uncultured bacteria that are metabolic responders in a microbiota	Massively parallel single-cell genome sequencing technique (SAG-gel Platform), 16S rRNA gene sequencing of V3-V4 using Illumina Miseq 2 x 300bp platform and bioinformatics (QIIME2 v.2019.1). Determination of the concentration of SCFA was done by GC-mass spectrophotometry	Functions of uncultured bacteria in the microbiota were elucidated	[34]
Study of human gut colonization linked to in utero by microbial communities in the amniotic fluid and placenta	Culture, Gradient Gel Electrophoresis (DGGE), 16S rRNA gene pyrosequencing of V1-V3 region, quantitative PCR and bioinformatics (PICRUST, QIIME, LEfSe)	The microbiota composition of infant gut at the age of 3-4 days begins to look like that detected in colostrum hence, the presumption that colonization is initiated prenatally by a distinct microbiota in the amniotic fluid and placenta	[35]

**Table 2.** Summary of the Potential Clinical Application of Various Technologies and Their Advantages and Disadvantages.

Technology/Methodology	Advantage	Disadvantage	Potential Clinical Application
Metagenomics (High-through sequencing)	<ul style="list-style-type: none"> <li>· Provides information on culturable and 'non-culturable' or yet to be cultured microorganisms.</li> <li>· Captures both viable and unviable species of microorganisms.</li> <li>· Essential details of diversity and community structure of the gut microbiota is provided</li> </ul>	<ul style="list-style-type: none"> <li>· Further studies on microorganisms present in the microbiota is not possible since direct extraction of DNA is employed restricting physical access to the microorganisms.</li> </ul>	<ul style="list-style-type: none"> <li>· Could be used by clinicians for the proper diagnosis of gastrointestinal diseases with overlapping clinical presentation. Or for identifying microbiological markers that predict the presence of certain diseases.</li> </ul>
High-throughput Metabolomics	<ul style="list-style-type: none"> <li>· Provides information on the various metabolites of gut resident microorganisms and how it correlates to disease conditions.</li> <li>· Specific metabolites identified could serve as biomarkers</li> <li>· Can be used for measuring and evaluating the effect of dietary intake on the gut microbiota</li> </ul>	<ul style="list-style-type: none"> <li>· Loss of metabolites of some members of the microbiota due to sample handling.</li> <li>· Drawback in its use for personalized medicine/nutrition because of the existence of variability in human microbiota and their metabolites.</li> </ul>	<ul style="list-style-type: none"> <li>· Monitoring metabolites of gut microbiota using high-throughput metabolomics can help in the early diagnosis and management of metabolic syndromes that has been linked with the gut microbiota.</li> <li>· Can guide physicians on recommending dietary intake to patients.</li> </ul>
High-throughput Metatranscriptomics	<ul style="list-style-type: none"> <li>· Captures active members of the microbiota</li> <li>· Gives insight into the functions of various members of the gut microbiota</li> <li>· Can provide information on how members of the microbiota respond to changes within their environment</li> </ul>	<ul style="list-style-type: none"> <li>· Since RNA is not as stable as DNA, handling of sample can results in biases in final results analyzed.</li> <li>· There is still a shortfall in metadata in repositories to which the enormous data generated from metatranscriptomics of the gut can match since this technology is still evolving</li> </ul>	<ul style="list-style-type: none"> <li>· Can identify how the function of a microbe in the gut influence the severity or progression of a disease</li> <li>· Can be used to monitor the interaction of the gut microbiota and host's mucosal immune system</li> </ul>

<p>Microfluidics</p>	<ul style="list-style-type: none"> <li>· Provide miniaturized platform for in vitro simulation, cultivation and manipulation of gut microbiota.</li> <li>· Make possible selective targeting and culture of important members of the gut microbiota.</li> <li>· Permit the combination of culture, DNA extraction, amplification and sequencing on a single platform.</li> </ul>	<ul style="list-style-type: none"> <li>· Human gut on chip might not give optimal performance as in natural human gut.</li> </ul>	<ul style="list-style-type: none"> <li>· This technology can be deployed clinically to monitor perturbation of gut microbiota in good time and enable precision in intervention by manipulating and stimulating the growth of beneficial or essential gut health promoting bacteria.</li> <li>· Microfluidics in microbiome studies can guide in the prescription of antibiotics.</li> </ul>
<p>High-throughput Culturing</p>	<ul style="list-style-type: none"> <li>· Culture gives access to the in-depth study of individual microorganisms that are cultured from the gut microbiota providing information on structure, morphology, physiology, growth conditions, inter &amp; intra species interactions.</li> <li>· Culture captures only viable bacteria population.</li> <li>· Enable enumeration of bacteria species present</li> </ul>	<ul style="list-style-type: none"> <li>· Laborious and time consuming.</li> <li>· Limited number of members of the microbiota are accounted for since majority of them are 'non-culturable' or yet to be cultured.</li> </ul> <p>Technique may be expensive due to the array of materials and specialized laboratory needed.</p>	<ul style="list-style-type: none"> <li>· Could provide avenue for precise treatment of gut diseases resulting from dysbiosis of specific species of bacteria and enable formulation of probiotics</li> </ul>

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## Keywords

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