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# CURRENT KNOWLEDGE ABOUT THE IMPLICATION OF BACTERIAL MICROBIOTA IN HUMAN HEALTH AND DISEASE

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***Abstract.*** *Recent advances in molecular genetics and the invention of new technologies led to a development in our knowledge about human microbiota, specifically bacterial one. The microbiota plays a fundamental role in the immunologic, hormonal and metabolic homeostasis of the host. After the initiation of the Human Microbiome Project, it became clear that the human microbiota consists of the 10-100 trillion symbiotic microbial cells harbored by each person, primarily bacteria in the gut, but also in other spots as the skin, mouth, nose, and vagina. Despite of the differences in studying bacterial species, decreased bacterial diversity and persistence has been connected with several diverse human diseases primarily diabetes, IBD (inflammatory bowel disease) and others; attempts were made even to explain psychiatric pathology. Several species emerged as dominant and were clearly linked to certain disorders or accepted as biomarkers of others. The current review aims to discuss key issues of our current knowledge about bacteria in human, the difficulties and methods of its analysis, its contribution to human health and responsibility for human diseases.*

***Key words:*** *microbiome, microbiota, bacteria, human diseases*

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Human beings live with trillions of microbes which coexist with and on them. The human microbiota refers to microorganisms that inhabit the epithelial barriers of our body, such as our skin, respiratory tract, and especially the gastrointestinal (GI) tract. The colon and rectum at the end of the gastrointestinal (GI) tract contain the highest microbial density in the human body [1]. This complex ecosystem is mainly composed of bacteria; the remainder includes viruses, archae, protozoa, and fungi [2]. Common bacterial phyla in the microbiota includes: Bacteroides, Firmicutes, Proteobacteria and Actinobacteria, comprising > 1000 prevalent bacterial species to confer a common core in the metagenome [3, 4].

The advantages of human microbiota on the host organism are numerous. The microbiota in the gut has been considered to be an "essential organ" [5]. It has the potential to increase energy extraction from food [6], nutrient harvest [9, 10], and alter appetite signaling [7, 8]. In addition, a large amount of metabolic processes of the microbiota are beneficial to the host, like the metabolism of undigested carbohydrates and the biosynthesis of vitamins [9]. The human microbiota also provides a physical barrier, protecting its host against foreign pathogens

through competitive exclusion and production of antimicrobial substances [10, 11]. Finally, the microbiota is essential in the development of the intestinal mucosa and immune system of the host [12, 13].

Collectively, the entire microbial genome is about – 150 times larger than our own. All these microorganisms participate in various physiological processes in the human body as food digestion [3], energy metabolism [14, 15], maturation of the immune cells [16], and provision of resistance [17]. Bacteria in the GIT follow a specific gradient of bacterial load being highest in the colon, lower in the duodenum and jejunum and lowest in the stomach. Alterations in microbial load are manifested in different types of human diseases, such as infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, and autoimmune diseases [18].

## DIFFICULTIES IN STUDYING MICROBIOTA

Next-generation metagenomic sequencing (NGS) methods offer a possibility to sequence all available genomes in a sample. This is due to the shotgun methodology in which the generated short fragments are subsequently assembled and reconstructed into contigs. This approach allows all genes from all organisms present in a sample to be analyzed, including microorganisms that cannot be easily cultured. This gives a new perspective to our understanding of the microbial pathogenesis in diseases.

The first metatranscriptomic analysis of the healthy human gut microbiota was performed in 2011 [19]. The most common approach to study bacteria is its 16S ribosomal RNA (rRNA) sequencing [20]. 6S rRNA gene is organized in an operon and is transcribed with into an rRNA subunit [21]. Being universally present in all bacteria and evolutionally conserved over time, sequencing of this gene provides a relatively easy approach for studying bacterial phylogeny and taxonomy. The analysis of 16S transcripts showed the phylogenetic structure of the active microbial community. Lachnospiraceae, Ruminococcaceae, Bacteroidaceae, Prevotellaceae, and Rickenellaceae were the predominant families detected in the active microbiota. A transcriptome analysis of bacteriophage communities in the periodontal microbiota performed later by RNAseq correlated the high microbial expression with the periodontal health [22].

Unfortunately, most gut microbes are currently uncultivable. Even with the use of recent technologies, 16S rDNA high-throughput sequencing identifies only approximately half of the bacterial species [23]. Therefore, other technologies are tried and developed in order to identify and isolate these microorganisms.

A chip-based isolation device (the iChip) was specifically designed to identify uncultivable microbes within complex microbial ecosystems [24]. The iChip is composed of hundreds of miniature diffusion chambers, each of which is inoculated with a single environmental cell. The capacity for microbial recovery using the iChip is many times higher than that of standard cultivation. A new device for in situ cultivation (the I-tip) was subsequently developed. The principal of the I-tip is similar to that of the iChip; however, the I-tip traps individual microbes within a gel. The in situ isolation of microbes from invertebrate organisms using the I-tip has recovered isolates from 34 novel microbial species [25].

The use of traditional animal models in microbiome studies continues to be widely applied but is limited as it cannot fully predict the results obtained in humans. There are two methods of in vitro simulation based on stem cells: the gut-on-a-chip system and colonic stem cell construction. The “gut-on-a-chip” systems were developed to study the complex crosstalk occurring within the gut microbiome. The gut-on-chip system is in vitro living cell-based model of the intestine, it is composed of microfluidic channels and a porous flexible membrane coated with an extracellular matrix and lined with human intestinal epithelial cells [26]. Microfluidic devices can also be used to study microbe-microbe interactions by traditional capillary-based assays [27].

Colonic stem cell construction is a recently developed in vitro system that is used to grow 3D organoid colonic epithelium [28]. Within a Matrigel overlay, spherical 3D structures are grown from colonic stem cells or intestinal stem cells as organoids. They contain various differentiated cell types, and mimic a barrier similar to that of intestinal or colonic epithelia [29, 30]. These organoids have recently been used to demonstrate that the Salmonella enterica serovar Typhimurium can successfully invade the epithelial cell layer [30], and that C. difficile can disrupt the epithelial barrier function [31].

## HUMAN MICROBIOTA IN HEALTH AND DISEASE

The body contains at least 1000 different species of known bacteria and carries 150 times more microbial genes than are found in the entire human genome [2]. The human microbiota affects host physiology to a great extent. Microbiotic composition and function differ according to different locations, ages, sexes, races, and diets of the host [27]. Commensal bacteria colonize the host shortly after birth. This simple community gradually develops into a highly diverse ecosystem during host growth [28].

Over time, host-bacterial associations have developed into beneficial relationships. Symbiotic bacteria

metabolize indigestible compounds, supply essential nutrients, defend against colonization by opportunistic pathogens [29]. For example, the intestinal microbiota is involved in the digestion of certain foods that cannot be digested by the stomach and small intestine, like dietary fibers (xyloglucans), which are commonly found in vegetables and can be digested by a specific species of Bacteroides [30]. Other non-digestible fibers, such as fructooligosaccharides and oligosaccharides, can be utilized by beneficial microbes, such as Lactobacillus and Bifidobacterium [31]. Studies have clarified the role of the gut microbiota in lipid and protein homeostasis as well as in the microbial synthesis of essential nutrient vitamins [32]. The gut microbiota has been shown to deliver vitamins to the host, such as folates, vitamin K, biotin, riboflavin (B2), cobalamin (B12), and possibly other B vitamins. In addition, gut-colonizing bacteria stimulate the normal development of the humoral and cellular mucosal immune systems [37]. A report has demonstrated that the gut microbiota generates a tolerogenic response that acts on gut dendritic cells and inhibits the type 17 T-helper cell (Th17) anti-inflammatory pathway [40]. However, not all microbiota lead to health benefits. Some induce inflammation under certain conditions.

### *Gastric, colorectal and esophageal cancers*

Apart from inherent genetic factors (such as mutations in oncogenes and tumor suppressor genes), numerous studies have revealed the important influence of microorganisms to cancer biology. Infectious agents such as *Helicobacter pylori*, hepatitis B and C viruses, and human papillomaviruses have been recognized as carcinogenic agents [32]. Some studies report an existing communication between the microorganisms in cancer and the surrounding tumor environment. Xuan C et al. noticed a dramatic reduction in bacterial load in breast tumor compared to healthy breast tissue and suggested that it could be an additional indicator used in the establishment of the diagnosis or staging of breast cancer [33]. It could be therefore speculated that a decrease in bacterial load in a healthy individual may be a signal of an elevated breast cancer risk. Relationships between gut microbiota and cancer are multifactorial and bidirectional [33].

*Helicobacter pylori* is another important bacterium that infects the stomach and contributes to the occurrence of gastric cancer, chronic gastritis, peptic ulcers and mucosa-associated lymphoid tissue lymphoma. It is well established that it acts by activating oncogenic signal transduction pathways (Ras-ERK -PI3K) to alter cell proliferation (through virulence factors (vacuolating cytotoxin A (VacA) and cytotoxin-associated gen A (CagA)), as well as by causing a continued oxidative stress within the tissue. Other microorganisms, besides *H.pylori*, could also cause gastric cancer. In human, members of Proteobacteria, Firmicutes, Actinobacteria and Fusobacteria phyla can be readily detected in samples of gastric cancer patients [34, 35]. On the contrary, only 3% of *H. pylori* infected people develop cancer [36], implying the importance of other phyla in gastric carcinogenesis. Gastric cancer microbiota increase nitrate reductase and nitrite reductase functions, potentially leading to more N-nitroso compounds that are carcinogenic [37]. Different groups observed a change in gastric microbiota in different stages of gastric carcinogenesis [38]: a significant enrichment of 21 bacterial taxa and depletion of 10 others. Some of these taxa correspond to *Peptostreptococcus stomatis, Parvimonas micra, Fusobacterium nucleatum* and some other bacteria, which are members of the human oral microbiota.

Colorectal cancer is the third most common cancer in the world with a high level of heritability that accounts for up to 35% [39], whereas about 5% of the cancer occur as a part of a known genetic predisposition syndrome [40]. The bacterial population of the colon consisted mainly of *Firmicutes* and *Bacteroidetes* [41]. Less abundant phyla in the colorectum include *Proteobacteria, Actinobacteria, Fusobacteria* and *Verrucomicrobia*. An important finding is that the stool transfer from colorectal cancer patients to two different mice models could promote intestinal carcinogenesis [42]. This demonstrated the carcinogenic properties of the colorectal cancer microbiota. Functional studies suggested that several bacteria, including *Bacteroides fragilis, Escherichia coli* and *Peptostreptococcus anaerobius*, may promote colorectal carcinogenesis, inducing direct DNA damage [43] and cholesterol biosynthesis [60]. The colorectal cancer microbiota exhibits dysbiosis with reduced overall bacterial diversity. Nevertheless, there is a significant enrichment in several genera as *Fusobacterium, Peptostreptococcus, Parvimonas* and *Porphyromonas* [44]. Similarly to gastric cancer, many of these bacteria are known members of the oral microbiota, present in the Human Oral Microbiome Database and are associated with periodontal diseases [45]. This suggests that oral-gut translocation of the microbiota can be associated with intestinal carcinogenesis.

The ***alpha bug hypothesis in cancer*** [46] states that certain oncogenic bacteria may remodel the microbial community to alter mucosal microenvironment and lead to cancer formation [46, 47]. There is a relationship between oral microbiota, periodontal disease and cancers [32]. In human, periodontal disease has been reported to be associated with cancers of the pancreas [48] and colorectum [49], head and neck carcinoma [50], lung cancer [51], breast

cancer [52]. Specific members of the oral microbiota including Porphyromonas gingivalis were associated with increased risk of pancreatic cancer [53, 54].

Chronic inflammation caused by gastroesophageal reflux (GER) is closely related to esophageal adenocarcinoma (EA) [55]. Regional differences in its incidence appear to be correlated with economic development. Long-term and frequent antibiotic exposures worldwide may lead to a higher incidence of GERD, thus resulting in an increasing morbidity from EA [56]. There were no detectable changes between local microbiomes of squamous cell carcinoma and adenocarcinoma [57]. A series of case-control studies suggested that *H. pylori* may play a protective role in the development of GERD and be associated to EA. However, the eradication of *H. pylori* does not worsen GERD or increase new GERD [58].

### *Metabolic, allergic and psychiatric diseases*

#### *A. Metabolic diseases*

The dysbiosis of intestinal flora affects the production of immune mediators and induces chronic inflammation, as well as metabolic dysfunction [59]. The interactions between the gut microbiota and host genotype may be crucial factors for obesity and related metabolic disorders [60]. Diet can modulate the composition and function of microbes in humans [61]. For example, a mouse study revealed that mice that were fed with lard for 11 weeks exhibited increased Toll-like receptor activation and white adipose tissue inflammation, along with reduced insulin sensitivity, compared with mice that were fed with fish oil [62]. The gut microbiota influences the circadian clock and undergoes circadian oscillations [63]. Disruption of the host circadian clock induces dysbiosis, which is associated with host metabolic disorders [64]. Growing numbers of studies indicate that an altered gut microbiome characterized by lower diversity is associated with diabetes. A study demonstrated that the human gut microbiome may induce insulin resistance [65] through species such as *Prevotella copri* and *Bacteroides vulgates*. The gut microbiota may directly affect T2D through its effect on the metabolism of amino acids; thus, future antidiabetic treatment strategies may target bacterial strains that cause imbalances in amino acid metabolism [66].

A reduced taxonomic or functional diversity of the microbiome was often observed in association with poorer health outcomes or disease in general [67]. Individuals suffering from metabolic liver disease exhibit microbiota instability in general. Specifically, fatty liver disease is linked to a long-term increase in opportunistic pathogens like *Enterobacteriaceae* or *Escherichia/Shigella*.

#### *B. Allergic diseases*

Bunyavanich et al. [68] examined the association between early-life gut microbiota and the resolution of cow's milk allergy. According to the results, early infancy (between age 3 and 6 months) is a window during which gut microbiota may shape food allergy outcomes in childhood. Bacterial taxa within Clostridia and Firmicutes could be studied as probiotic candidates for milk allergy therapy. The gut microbiota interacts with the immune system intimately, providing signals to promote the maturation of regulatory antigen-presenting cells (APCs) and regulatory T cells (Tregs), which play a crucial role in the development of immunological tolerance. The specific members of the microbiota, such as Clostridium species, interact with Treg and regulate immunoglobulin E (IgE) levels [69].

Food allergy (FA) is associated with the intestinal microbiota composition of infants [70]. There are several key FA-associated bacterial phylotypes, which are significantly changed in infancy and are positively associated with the development of FA. The proportion of abundant Bacteroidetes, Proteobacteria, and Actinobacteria phyla were significantly reduced, while the Firmicutes phylum was highly enriched in the FA group. The genera Enterococcus and Staphylococcus are FA-enriched phylotypes which negatively correlate with interleukin-10 [70].

#### *C. Psychiatric diseases*

The gut-brain axis plays a key role in psychiatric diseases and its normal functioning maintains the normal brain and GI function. Depression is accompanied by increased IgM and IgA responses directed against gram negative gut commensals including *Hafnia alvei, Pseudomonas aeruginosa, Morganella morganii, Pseudomonas putida, Citrobacter koseri*, and *Klebsiella pneumonia* [71]. The average values of serum IgM and IgA against LPS of these commensals were significantly higher in chronically depressed patients than in those with melancholia or recurrent depression. The authors conclude that the "translocated" gut commensal bacteria activate immune cells to produce IgA and IgM responses and that this phenomenon may play a role in the pathophysiology of chronic depression [71].

Gut microbiota regulates neurotransmitter signals. Common neurotransmitters include serotonin (5-HT), noradrenaline, dopamine, and gamma-aminobutyric acid (GABA), which have marked effects on the brain and behavior [72]. The gut microbiota can regulate both the expression of central neurotransmitters and related receptors, as well as the peripheral neurotransmitter levels. Germ-free (GF) mice showed different alteration of 5-HT, noradrenaline, dopamine, and related receptors in different areas of the brain [73], as well as decreased serotonin in the peripheral nervous system and intestine [74]. Although some gut neurotransmitters – such as 5-HT, GABA, and dopamine – cannot cross the BBB (blood-brain-barrier), these gut neurotransmitters may act on the vagus nerve or affect periphery signaling, thereby influencing brain functions [75].

Gut microbiota dysbiosis in early years of life before three years of age can result in neurodevelopmental diseases such as autism spectrum disorder (ASD) [76]. The pathophysiology of autism is not fully clear. However, children with ASD often have GI problems, such as indigestion, poor absorption, overgrowth of intestinal pathogenic bacteria and abnormal GI fistula [77]. Finegold et al. [78] reported that in patients with severe autism, Bacteroidetes and Actinobacteria phyla were at a higher level, while Firmicutes and Proteobacteria phyla were more abundant in the healthy individual. Desulfovibrio species and *Bacteroides vulgatus* in the feces of autistic children were significantly higher than the healthy controls. Moreover, Wang et al. [79] confirmed that Bifidobacteria species and *Akkermansia muciniphila* in autistic children were at lower relative abundances when compared to the control.

### *Gut microbiota can modulate drug efficacy, and used as a biomarker of patients' outcomes*

The gut microbiota has been shown to modulate anticancer drug efficacy. Altered gut microbiota is associated with resistance to chemo drugs or immune checkpoint inhibitors (ICIs), whereas supplementation of distinct bacterial species restores responses to the anticancer drugs.

Moreover, microbial shifts are highly distinct across tumor stages [80, 81]. Bacteria have been reported to promote inflammation and cancer cell proliferation via cytokine production. In addition to its function in modulating inflammation, the microbiota influences the efficacy of cancer treatment. Preclinical data from a colon cancer mouse model identified Gammaproteobacteria as the key bacteria that metabolise gemcitabine, a chemotherapeutic drug, into its inactive form via cytidine deaminase. Human pancreatic ductal adenocarcinoma (PDAC) contains elevated level of Gammaproteobacteria compared to the normal pancreas. The detection of Gammaproteobacteria may explain the gemcitabine resistance in PDAC patients and targeting these bacteria may sensitize tumor to gemcitabine treatment [82].

Microbiota could mediate chemotherapy induced toxicity. Mice depleted in gut microorganisms after treatment with broad-spectrum antibiotics were more susceptible to methotrexate induced small intestinal injury. Gastrointestinal toxicity, causing diarrhoea, pain and weight loss, is a common adverse effect of chemotherapy, resulting in morbidity and mortality [83]. Such a drug is irinotecan which causes severe diarrhoea in up to 40% of patients, requiring dose reduction and, in many cases, premature termination of the drug [84]. Its administration increases the abundance of caecal Clostridium cluster XI and Enterobacteriaceae, both of which are potentially pathogenic [84].

Irinotecan's active metabolite, SN-38, is converted in the liver to an inactive glucuronide (SN-38G). Wallace and colleagues [85] showed that bacterial β-glucuronidases cleaves the glucuronide moiety for use as a carbon source, releasing the active SN-38 metabolite into the bowel lumen, generating diarrhoea. All these experiments show that gut microbiota could be potentially targeted to improve efficacy and reduce the toxicity of current chemotherapy agents.

Dysbiotic states seem to be associated with poor or deleterious outcomes from chemotherapy so clinicians need urgently biomarkers of dysbiosis as predictors of patients' outcomes. They have to reflect the bacterial variation in tumour stage and phenotype. Such an example is *Fusobacterium nucleatum* which is associated with advanced stage and poor outcome in patients with colorectal cancer [86]. Montassier and colleagues [87] found that a diverse gut microbiota is associated with protection against chemotherapy-related bloodstream infection. The authors suggested a microbiota-based predictive *risk index model*, which might be used to stratify patients at risk of complications before treatment. Generally, the reduced microbial diversity over the course of chemotherapeutic treatment corresponds to higher rate of infection within 90 days in patients with acute myeloid leukaemia [88].

## CONCLUSION

The past two decades appeared to be very fruitful in understanding the impact of the human microbiota on the host health and disease, and more specifically the impact of its bacterial component. The role of decreased bacterial variety and increased homogeneity has been implicated in a plethora of human pathologies. Few papers subtly attempted to depict enriched bacterial species as biomarkers of certain disorders.

Despite the enormous progress that has been made, our knowledge of the specific bacterial microbiota members is out-of-date. The current review aimed to discuss key issues of our current knowledge about bacteria in human, the difficulties and methods of its analysis, its contribution to human health and responsibility for human diseases. The next steps will probably include the development of novel probiotic strains to treat diseases, improving bacterial microflora. But before that, scientific society has to be aware of the molecular-level details underpinning bacterial effects.

## REFERENCES


1. Gill SR, Pop M, Deboy RT et al. Metagenomic analysis of the human distal gut microbiome. Science. 2006; 312(5778):1355-1359.
2. Franzosa EA, Morgan XC, Segata N et al. Relating the metatranscriptome and metagenome of the human gut. Proc Natl Acad Sci. 2014; 111(22):E2329.

3. Qin J, Li R, Raes J et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010; 464(7285):59-65.
4. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016; 14(8):e1002533.
5. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO reports 2006; 7(7):688-693.
6. Turnbaugh PJ, Ley RE, Mahowald MA. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444(7122):1027-1031.
7. Cani PD, Dewever C, Delzenne NM. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br. J. Nutr. 2004 Sep;92(3):521-6. doi: 10.1079/bjn20041225.
8. Perry RJ, Peng L, Barry NA et al. Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. Nature 2016; 534(7606):213-217.
9. Roberfroid MB, Bornet F, Bouley C et al. Colonic microflora: nutrition and health. Summary and conclusions of an International Life Sciences Institute (ILSI) [Europe] workshop held in Barcelona, Spain. Nutrition Reviews. 1995; 53(5):127-130.
10. Cash HL, Whitham CV, Behrendt CL et al. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science. 2006; 313(5790):1126-1130.
11. Schauber J, Svanholm C, Termen S et al. Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways. Gut. 2003; 52(5):735-741.
12. Bouskra D, Brezillon C, Berard M et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. Nature 2008; 456(7221):507-510.
13. Rakoff-Nahoum S, Medzhitov R. Innate immune recognition of the indigenous microbial flora. Mucosal Immunol, 2008,1, S10–S14.
14. Turnbaugh PJ, Ley RE, Mahowald MA et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444(7122):1027-1031.
15. Karlsson F, Tremaroli V, Nielsen J et al. Assessing the human gut microbiota in metabolic diseases. Diabetes. 2013; 62(10):3341-3349.
16. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012; 336(6086):1268-1273.
17. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat. Rev. Immunol. 2013; 13(11):790-801.
18. Wang B, Yao M, Lv L et al. The Human Microbiota in Health and Disease. Engineering 2017; 3(1):71-82.
19. Gosalbes MJ, Durban A, Pignatelli M et al. Metatranscriptomic approach to analyze the functional human gut microbiota. PloS one 2011; 6(3):e17447.
20. Olsen GJ, Lane DJ, Giovannoni SJ et al. Microbial ecology and evolution: a ribosomal RNA approach. Annual Review of Microbiology. 1986; 40:337-365.
21. Schlessinger D, Ono M, Nikolaev N et al. Accumulation of 30S preribosomal ribonucleic acid in an Escherichia coli mutant treated with chloramphenicol. Biochemistry. 1974; 13(21):4268-4271.
22. Santiago-Rodriguez TM, Naidu M, Abeles SR et al. Transcriptome analysis of bacteriophage communities in periodontal health and disease. BMC Genomics. 2015; 16:549.
23. Arnold JW, Roach J, Azcarate-Peril MA. Emerging Technologies for Gut Microbiome Research. Trends Microbiol. 2016; 24(11):887-901.
24. Nichols D, Cahoon N, Trakhtenberg EM et al. Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species. Applied and Environmental Microbiology. 2010; 76(8):2445-2450.
25. Jung D, Seo EY, Epstein SS et al. Application of a new cultivation technology, I-tip, for studying microbial diversity in freshwater sponges of Lake Baikal, Russia. FEMS Microbiology Ecology 2014; 90(2):417-423.
26. Kim HJ, Huh D, Hamilton G et al. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab on a chip 2012; 12(12):2165-2174.
27. Englert DL, Manson MD, Jayaraman A. Investigation of bacterial chemotaxis in flow-based microfluidic devices. Nature Protocols. 2010; 5(5):864-872.
28. Wang Y, Ahmad AA, Sims CE et al. In vitro generation of colonic epithelium from primary cells guided by microstructures. Lab on a chip 2014; 14(9):1622-1631.
29. Gracz AD, Williamson IA, Roche KC et al. A high-throughput platform for stem cell niche co-cultures and downstream gene expression analysis. Nat. Cell Biol. 2015; 17(3):340-349.
30. Forbester JL, Goulding D, Vallier L et al. Interaction of Salmonella enterica Serovar Typhimurium with Intestinal Organoids Derived from Human Induced Pluripotent Stem Cells. Infection and Immunity. 2015; 83(7):2926-2934.
31. Leslie JL, Huang S, Opp JS et al. Persistence and toxin production by Clostridium difficile within human intestinal organoids result in disruption of epithelial paracellular barrier function. Infection and Immunity. 2015; 83(1):138-145.
32. Elgueta MF, Echevarria GC, De la Fuente N et al. Effect of intravenous fluid therapy on postoperative vomiting in children undergoing tonsillectomy. Br J Anaesth. 2013; 110(4):607-614.
33. Xuan C, Shamonki JM, Chung A et al. Microbial dysbiosis is associated with human breast cancer. PloS one. 2014; 9(1):e83744.
34. Aviles-Jimenez F, Vazquez-Jimenez F, Medrano-Guzman R et al. Stomach microbiota composition varies between patients with non-atrophic gastritis and patients with intestinal type of gastric cancer. Scientific Reports 2014; 4:4202.
35. Dicksved J, Lindberg M, Rosenquist M et al. Molecular characterization of the stomach microbiota in patients with gastric cancer and in controls. J. Med. Microbiol. 2009; 58(Pt 4):509-516.
36. Peek RM, Jr., Crabtree JE. Helicobacter infection and gastric neoplasia. J Pathol. 2006; 208(2):233-248.
37. Correa P: Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Research. 1992, 52(24):6735-6740.
38. Coker OO, Dai Z, Nie Y et al. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut 2018; 67(6):1024-1032.
39. Lichtenstein P, Holm NV, Verkasalo PK et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med. 2000; 343(2):78-85.
40. Foulkes WD. Inherited susceptibility to common cancers. N Engl J Med. 2008; 359(20):2143-2153.
41. Arumugam M, Raes J, Pelletier E et al. Enterotypes of the human gut microbiome. Nature 2011; 473(7346):174-180.
42. Wong SH, Zhao L, Zhang X et al. Gavage of Fecal Samples From Patients With Colorectal Cancer Promotes Intestinal Carcinogenesis in Germ-Free and Conventional Mice. Gastroenterology 2017; 153(6):1621-1633 e1626.
43. Cuevas-Ramos G, Petit CR, Marcq I et al. Escherichia coli induces DNA damage in vivo and triggers genomic instability in mammalian cells. Proceedings of the National Academy of Sciences 2010; 107(25):11537.
44. Dai Z, Coker OO, Nakatsu G et al. Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. Microbiome 2018; 6(1):70.
45. Drewes JL, White JR, Dejea CM et al. High-resolution bacterial 16S rRNA gene profile meta-analysis and biofilm status reveal common colorectal cancer consortia. NPJ Biofilms Microbiomes 2017; 3:34.
46. Abreu MT, Peek RM, Jr.. Gastrointestinal malignancy and the microbiome. Gastroenterology 2014; 146(6):1534-1546 e1533.
47. Sears CL, Pardoll DM. Perspective: alpha-bugs, their microbial partners, and the link to colon cancer. J Infect Dis. 2011; 203(3):306-311.

48. Bracci PM. Oral Health and the Oral Microbiome in Pancreatic Cancer: An Overview of Epidemiological Studies. Cancer J. 2017; 23(6):310-314.
49. Ahn J, Segers S, Hayes RB. Periodontal disease, Porphyromonas gingivalis serum antibody levels and orodigestive cancer mortality. Carcinogenesis 2012; 33(5):1055-1058.
50. Tezal M, Sullivan MA, Hyland A et al. Chronic periodontitis and the incidence of head and neck squamous cell carcinoma. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2009; 18(9):2406-2412.
51. Mai X, LaMonte MJ, Hovey KM et al. History of periodontal disease diagnosis and lung cancer incidence in the Women's Health Initiative Observational Study. Cancer Causes Control : CCC 2014; 25(8):1045-1053.
52. Freudenheim JL, Genco RJ, LaMonte MJ et al. Periodontal Disease and Breast Cancer: Prospective Cohort Study of Postmenopausal Women. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2016; 25(1):43-50.
53. Farrell JJ, Zhang L, Zhou H et al. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. Gut 2012; 61(4):582.
54. Fan X, Alekseyenko AV, Wu J et al. Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study. Gut 2018; 67(1):120.
55. Lagergren J, Bergstrom R, Lindgren A et al. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med. 1999; 340(11):825-831.
56. Pei Z, Pei Z, Bini EJ et al. Bacterial biota in the human distal esophagus. Proc Natl Acad Sci U S A. 2004;101(12):4250-4255. doi:10.1073/pnas.0306398101
57. Finlay IG, Wright PA, Menzies T et al. Microbial flora in carcinoma of oesophagus. Thorax 1982; 37(3):181-184.
58. Hamada H, Haruma K, Mihara M et al. High incidence of reflux oesophagitis after eradication therapy for *Helicobacter pylori*: impacts of hiatal hernia and corpus gastritis. Aliment Pharmacol Ther. 2000; 14(6):729-735.
59. Sommer F, Backhed F. The gut microbiota--masters of host development and physiology. Nature Reviews Microbiology 2013; 11(4):227-238.
60. Le Chatelier E, Nielsen T, Qin J et al. Richness of human gut microbiome correlates with metabolic markers. Nature 2013; 500(7464):541-546.
61. David LA, Maurice CF, Carmody RN et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014; 505(7484):559-563.
62. Caesar R, Tremaroli V, Kovatcheva-Datchary P et al. Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling. Cell Metabol. 2015; 22(4):658-668.
63. Leone V, Gibbons SM, Martinez K et al. Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. Cell Host Microbe 2015; 17(5):681-689.
64. Thaiss CA, Zeevi D, Levy M et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Cell 2014; 159(3):514-529.
65. Pedersen HK, Gudmundsdottir V, Nielsen HB et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature 2016; 535(7612):376-381.
66. Forslund K, Hildebrand F, Nielsen T et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature 2015; 528(7581):262-266.
67. Frost F, Kacprowski T, Rühlemann M, et al. Long-term instability of the intestinal microbiome is associated with metabolic liver disease, low microbiota diversity, diabetes mellitus and impaired exocrine pancreatic function. Gut. 2021; 70(3):522-530. doi: 10.1136/gutjnl-2020-322753.
68. Bunyavanich S, Shen N, Grishin A et al. Early-life gut microbiome composition and milk allergy resolution. J Allergy Clin Immunol 2016; 138(4):1122-1130.
69. Atarashi K, Tanoue T, Shima T et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 2011; 331(6015):337-341.
70. Ling Z, Li Z, Liu X et al. Altered fecal microbiota composition associated with food allergy in infants. Appl Environ Microbiol. 2014; 80(8):2546-2554.
71. Maes M, Kubera M, Leunis JC et al. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. J Affect Disord. 2012; 141(1):55-62.
72. Rieder R, Wisniewski PJ, Alderman BL et al. Microbes and mental health: A review. Brain, Behavior, and Immunity 2017; 66:9-17.
73. Kennedy PJ, Murphy AB, Cryan JF et al. Microbiome in brain function and mental health. Trends in Food Science & Technology 2016; 57:289-301.
74. Yano JM, Yu K, Donaldson GP et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015; 161(2):264-276.
75. Barrett E, Ross RP, O'Toole PW et al. gamma-Aminobutyric acid production by culturable bacteria from the human intestine. J Appl Microbiol.2012; 113(2):411-417.
76. Wang C, Geng H, Liu W et al. Prenatal, perinatal, and postnatal factors associated with autism: A meta-analysis. Medicine 2017; 96(18):e6696.
77. Fond G, Boukouaci W, Chevalier G et al. The "psychomicrobiotic": Targeting microbiota in major psychiatric disorders: A systematic review. Pathologie-biologie 2015; 63(1):35-42.
78. Finegold SM, Dowd SE, Gontcharova V et al. Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 2010; 16(4):444-453.
79. Wang L, Christophersen CT, Sorich MJ et al. Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and Bifidobacterium spp. in feces of children with autism. Appl Environ Microbiol. 2011; 77(18):6718-6721.
80. Cheng WY, Wu CY, Yu J. The role of gut microbiota in cancer treatment: friend or foe? Gut 2020; 69(10):1867-1876.
81. Bachmann R, Leonard D, Delzenne N et al. Novel insight into the role of microbiota in colorectal surgery. Gut 2017; 66(4):738-749.
82. Geller LT, Barzily-Rokni M, Danino T et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 2017; 357(6356):1156-1160.
83. Paci A, Veal G, Bardin C et al. Review of therapeutic drug monitoring of anticancer drugs part 1 – Cytotoxics. Eur J Cancer 2014; 50(12):2010-2019.
84. Vanhoefer U, Harstrick A, Achterrath W et al. Irinotecan in the treatment of colorectal cancer: clinical overview. Journal of Clinical Oncology : official journal of the American Society of Clinical Oncology 2001; 19(5):1501-1518.
85. Wallace BD, Wang H, Lane KT et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science 2010; 330(6005):831-835.
86. Mima K, Nishihara R, Qian ZR et al. Fusobacterium nucleatum in colorectal carcinoma tissue and patient prognosis. Gut 2016; 65(12):1973-1980.
87. Montassier E, Al-Ghalith GA, Ward T et al. Pretreatment gut microbiome predicts chemotherapy-related bloodstream infection. Genome medicine 2016; 8(1):49.
88. Galloway-Pena JR, Smith DP, Sahasrabhojane P et al. The role of the gastrointestinal microbiome in infectious complications during induction chemotherapy for acute myeloid leukemia. Cancer 2016; 122(14):2186-2196.