From Regular Catharsis with Castor Oil to Recognizing the Importance of the Intestinal Microbiota

Carlo Romano Settanni^a Gianluca Ianaro^a Francesco Franceschi^b
Giovanni Gasbarrini^a Antonio Gasbarrini^a

^aDigestive Disease Centre, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Catholic University of Sacred Heart, Rome, Italy; ^bDepartment of Emergency, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Catholic University of the Sacred Heart, Rome, Italy

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Abstract

The need to shed light on the unknown aspects of pathophysiology of common disorders, such as gastrointestinal ones, has led researchers through last decades to study and define the role of microorganisms within the human intestine and their interactions with the host. The progress of technology has permitted the overcoming of culture-based methods to study microbes and paved the way to molecular techniques, which allow the analysis of microbial genome, microbial functions, and metabolism. These progresses opened a window on the world of microbiology and permitted to deepen into the key role played by gut microbiota and dysbiosis in health status and diseases, both gastrointestinal and extraintestinal. So, scientists focused their attention in developing new strategies to restore eubiosis and to manipulate gut microbes by modifying dietary habits, administering antibiotics, probiotics, and prebiotics and using fecal microbiota transplantation as treatment of gastrointestinal, infectious, cardiovascular, metabolic, immune-mediated,

neuro-psychiatric, and oncological disorders. The next challenges will be to elaborate standard protocols with definite outcomes predictors in disease-specific settings.

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Introduction

Gastrointestinal disorders have always had a very high prevalence in the world population and still today people frequently ask for a gastroenterological consultation because of them [1, 2]. As many other common disorders, they often tend to become chronic, symptoms relapse despite therapies, and the patients' quality of life and work productivity are significantly impaired [3–5]. The most usual are functional disorders, like irritable bowel syndrome (IBS) and functional dyspepsia, though the last decades have seen a rise of inflammatory bowel diseases (IBD), characterized by chronic inflammation of the intestine [5–7]. The burden of health care management related to these conditions is considerable as, in most cases, treatments contemplate a multidisciplinary approach, not only due to the complexity of the therapies but also because of the need to support the patients' global health,

including consultations from nutritionists, psychologists and psychiatrists, surgeons, and other specialists [2, 8, 9].

The knowledge of the pathophysiology of these diseases has increased through the years and it is still growing, and we are aware that they are due to multifactorial conditions; however, many sides of these mechanisms remain unclear even nowadays. Moreover, in most instances, current therapies only partially resolve symptoms and prevent disease relapse and progression, and we are far from identifying a definitive cure that targets the pathophysiological processes [10–13]. These pending issues led researchers and physicians to focus their attention through the ages on the role played by microbes within the host and, consequently, to study human interactions with microorganisms. Indeed, it was noted that both IBS and IBD may be triggered or exacerbated by an episode of infective acute gastroenteritis, which may cause persistent subclinical inflammation, altered gut motility and alteration of intestinal barrier function [14, 15]. Moreover, interventions aimed at modulating intestinal microbiota (diet, antibiotics, probiotics, prebiotics, and fecal microbiota transplantation) have been associated to an improvement of gastrointestinal symptoms in these conditions, even though with some still unclear aspects [16, 17].

Nowadays, thanks to the new technologies, which permit an extensive gut microbiota profiling, we can claim that, due to the vastity of roles played by intestinal microbes, including those regarding chemical transformations of nutrients, inflammatory and immunoregulatory effects, barrier and metabolic functions, human gut microbiota can be effectively considered as a real organ, whose equilibrium perturbances may set up disease promoting conditions [18]. Indeed, as microbes existed for billions of years before humans [19], it is logical to think that human beings coevolved with them symbiotically, creating a superorganism [20]. Over the years, it was revealed that gut microbiota homeostasis is crucial not only for gastrointestinal and global wellness but it is also involved in immune-mediated diseases, metabolic and cardiovascular disorders, oncological conditions, and neuro-psychiatric diseases [21].

Techniques to Study the Gut Microbiota

Time has passed since the seventeenth century, when the Dutch scientist Antony van Leeuwenhoek discovered “animalcules” and other microorganisms by the use of a microscope and started the revolution of microbiology [22]. Then, in the first decade of the twentieth century,

scientists began to use tissue samples for isolation of viruses [23] and, subsequently, bacterial cultures to identify single infective pathogens for the study of virulence and antibiotic susceptibility became available [24].

Lactulose and glucose breath tests spread in the second half of the 1,900 to diagnose small intestinal bacterial overgrowth and orocecal transit [25]. Until modern times, researchers were focused mainly on pathogenic microbes and the interest in studying nonpathogenic colonizing microorganisms came out when the evidences of their impact on human health and disease became evident [26].

The need of culture-independent techniques came up when the scientific community accumulated sufficient evidence to recognize the importance of studying commensal flora, as culture-dependent tests were limited by the impossibilities and difficulties of identifying some species, as in the case of obligate anaerobes [27]. In the 1990s, these necessities were solved by molecular techniques, as the amplicon sequencing of the 16S ribosomal RNA, a 1,500 nucleotide long sequences from small ribosomal subunits used as a genetic marker of diversity [28]. To identify the different bacterial species, this method studies the differences of nucleotides in the V1–V9 hypervariable regions by linking specific primers [27]. According to the similarities of the sequences, these are clustered into operational taxonomic units, which are related to the phylogenesis of the species. A more extensive approach is shotgun metagenomics sequencing, which allows to sample the whole genome of a microbial community by using random primers [29]. This method is more expensive and need more informatic resources, but it permits to elucidate the whole taxonomic composition of strains and the functions of a microbial system [30].

The introduction of next-generation sequencing in the 2000s, which allows simultaneous sequencing of a large number of small fragments of DNA, has led to an abatement of costs and to a significant reduction of sequencing time, and permitted the study of diverse microbiomes in their native environments [31, 32]. The Human Microbiome Project, a worldwide program, which has the main aim to characterize the whole human microbiome between different communities and its influence on physiology and disease, has made wide use of these techniques [33].

The progress of technology allowed to integrate the metagenomic analyses with the study of genes expression of bacterial communities by sequencing mRNA molecules, namely, metatranscriptomics [34]. This strategy can reveal details of transcriptionally active bacterial spe-

cies, obtaining information about the functional status of gut microbes. Another step ahead in understanding microbial functions and metabolism was made with the introduction of metaproteomics, which uses spectrometry to assess the whole amount of proteins in a certain sample [35], and metabolomics, that studies and quantifies the metabolome of a biological system, consisting of small molecules involved in chemical processes [36].

Together, the combination of “omic” techniques allows to admire the comprehensive panorama of composition and functions of both fecal and mucosal gut microbiota, and its interaction with the host [37].

Role of Gut Microbiota in Disease

Through the last decades, the knowledge about the complex interactions between microbes, human biology, and pathophysiology went far beyond the simple relation “one pathogen-one disease.” Considering the example of *Helicobacter pylori*, a renowned Gram-negative bacterium which is associated not only to peptic ulcer disease but also to gastric adenocarcinoma, gastric lymphoma, colon cancer and adenomatous polyps, sideropenic anemia, and immune thrombocytopenic purpura, it is easy to imagine how gut microbes may be involved in the development of many intestinal and systemic diseases [38, 39].

The microbiota harbored in humans is composed by viruses, fungi, protozoa, archaea, and mainly bacteria, which are estimated to be approximately 10^{13} cells in the whole body with a ratio of bacterial to human cells of 1:1 [40]. Bacterial micro-organisms are most represented in the gastrointestinal tract and mainly belong to the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* [41]. Dysbiosis is the loss of balance of microbial population, which implies a decrease in overall diversity with reduction of beneficial microbes and increase of pathobionts; it is caused by perturbing factors (such as diet, stressful events, infections, or antibiotics), and it is involved the developing of many diseases [42].

Primarily, a decrease in biodiversity and an increase of *Proteobacteria*, mainly *Escherichia coli*, have been widely associated with IBD [43, 44]. A reduction of the butyrate producing phyla *Firmicutes* and *Bacteroidetes* and a decrease of the ratio *Bacteroidetes*: *Firmicutes* was reported as well [45, 46]. Also *Fusobacterium* spp. has been isolated more frequently from tissue samples of IBD patients than healthy controls [47]. In treatment-naïve children with new onset of Crohn’s disease, an increase

of *Enterobacteriaceae*, *Pasteurellaceae*, *Veillonellaceae*, and *Fusobacteriaceae* and a reduction of *Erysipelotrichales*, *Bacteroidales*, and *Clostridiales* were reported on gastrointestinal tissue samples [48]. Ulcerative colitis (UC) patients show a decrease of *Akkermansia muciniphila*, *Butyricicoccus pullicaecorum*, *Clostridium colinum*, and *Roseburia* [49]. However, whether dysbiosis is a cause or a consequence of bowel inflammation, it still remains uncertain [43]. It is broadly accepted that IBDs arise from an intense inflammatory immune-mediated response to gut microbes in genetically predisposed individuals [50].

In general, patients with IBS have a reduced gut microbial diversity; furthermore, many studies show a common shift of microbiota composition, often distinguished by an increase of *Proteobacteria*, (especially *Enterobacteriaceae*) and *Firmicutes*, including *Lactobacillus* and *Ruminococcus* genera, frequently associated to a reduction of *Actinobacteria* (mainly bifidobacteria), *Faecalibacterium*, and *methanogenic* bacteria [51, 52].

As certain gut microbes have already been associated to specific cancers due to chronic inflammation mechanisms or genotoxicity, like in the cases of *H. pylori* in gastric cancer or *Salmonella typhi* in biliary cancer, the hypothesis of carcinogenetic mechanisms linked to dysbiosis has been postulated [53]. Indeed, there is an intensive bidirectional crosstalk between the gut microbiota and the host’s nervous system through hormones and active molecules that can affect tumorigenesis by the production of anticancer molecules or by triggering immune-mediated responses against tumor development, or, on the contrary, by promoting cancer with similar pathways [54]. As an example, *Fusobacterium nucleatum*, which has been associated with colon adenocarcinoma, may inhibit Natural Killer cells cytotoxicity and the activity of tumor infiltrating lymphocytes [55]. Moreover, *E. coli* strains producing the genotoxic compound colibactin, which has been identified in colorectal cancer biopsies, may enhance tumor growth by producing growth factors in preclinical models [56].

Dysbiosis has been widely associated to many autoimmune conditions, and the role of gut microbes in inducing the loss of immune tolerance in individuals with genetic susceptibility seems a key factor [57]. The finding of anti-*Saccharomyces cerevisiae* antibodies in antiphospholipid syndrome, systemic lupus erythematosus (SLE), and rheumatoid arthritis it’s a clue; indeed, they were found to have an overlapping structure with common autoantigens [58]. Several studies reported microbial imbalances in different immune-mediated pathologies: patients with

early rheumatoid arthritis show a significant reduction of bifidobacteria, *Bacteroides*, *Porphyromonas*, and *Prevotella* if compared to individuals suffering from fibromyalgia [59]; there is an increase of the relative abundance of *Proteobacteria* in SLE patients [60]; and patients with SLE or Sjögren's syndrome have a reduced bacterial richness, low *Firmicutes*:*Bacteroidetes* ratio, and an increase of *Bacteroides* in stools [61]; an imbalance in microbial diversity toward bifidobacteria, *Bacteroides*, and *E. coli* during young age may influence immune modulation and trigger susceptibility to type 1 diabetes mellitus [62].

Several clinical studies reported alterations of microbial population in metabolic syndrome and type 2 diabetes mellitus. For instance, it is described that *Firmicutes* are increased in lean compared to overweight males [63]. Besides, diabetic patients are characterized by a reduction of butyrate-producing bacteria (such as *Faecalibacterium prausnitzii* and *Roseburia intestinalis*), together with an increase of *E. coli*, *Eggerthella lenta*, and clostridia [64]. A reduction of microbial diversity has been associated with an increase of insulin resistance, inflammation, and adiposity.

Of interest, also cardiovascular diseases seem to have a relevant pathogenic substrate in gut microbial composition. *Enterobacteriaceae* and *Streptococcus* spp. are more represented in individuals with atherosclerosis [65], and the genus *Collinsella* is reported to be increased in symptomatic atherosclerosis cases [66]. Trimethylamine N-oxide, a product of microbial metabolism, has a role in atherogenesis [67], and it has been assessed that blood levels of this compound in acute coronary syndromes are a predictor of adverse cardiac events [68].

As it is known that a connection between gut microbiota and brain exists and that intestinal microbes are crucial in neurodevelopment and neurodegeneration, it is not surprising that neurological and psychiatric disorders have been linked to disturbances of microbial normal composition [69]. As an example, it has been reported that Prevotellaceae in stools of patients with Parkinson's disease are reduced and that the abundance of *Enterobacteriaceae* correlated with the grade of motor disturbances [70]. Moreover, an increase of genera *Clostridium* spp., *Desulfovibrio* spp., and *Lactobacillus* spp., a reduction of *Bifidobacterium* spp. and *Prevotella* spp., together with a decrease of the *Bacteroidetes*:*Firmicutes* ratio has been assessed in individuals with autism spectrum disorders [71–73]. Preclinical evidence shows that gut bacteria transplantation from depressed humans induces depressive symptoms in germ-free mice, but currently

data on humans about a precise association between major depression and specific microbial imbalance are still inconsistent [74].

Modulation of Gut Microbiota in Disease

Various factors may influence gut microbial composition, and the restoration of eubiosis through different strategies is supposed to be the key of resolution or amelioration of many diseases [75]. Considering the different abundance of gut microbes in different clinical conditions, specific modulations of the reduced or increased species may allow to manipulate the state of illness.

As previously mentioned, dietary habits are essential for the regulation of intestinal microbial populations, and changing our nutrition may exert microbiota-induced beneficial effects. For instance, a higher bacterial diversity with an increase of butyrate-producing microbes has been associated with Mediterranean diet, consumption of fat sources derived from vegetables and poly- and mono-unsaturated fats, which are generally linked to a healthy status [76]. A diet with low intake of fermentable oligo-, di-, mono-saccharides, and polyols, whose assumption can cause water retention and fermentation by gut microbes with hydrogen or methane production and distension of bowels, is reported to mitigate symptoms in patients with IBS, even if with a reduction of bifidobacteria and total bacterial abundance after a short-term fermentable oligo-, di-, mono-saccharides, and polyols restriction [77]. A study in a mouse model of pancreatic cancer assessed a decline of tumor growth and a change in gut microbial composition, with a reduction of the proinflammatory microbes (*Bacteroides acidifaciens*, *E. coli*, and others) and an increase of the anti-inflammatory butyrate-producing bacteria (mainly *Lachnospiraceae*), after a fiber-rich food regimen with resistant starch [78]. Another recent preclinical trial demonstrated that fasting-mimicking diet and refeeding in a mouse model of chronic colitis can improve IBD-associated symptoms and histology through modulating gut microbiota and other mechanisms [79]. In a near future, new evidences may pave the way for engineered diets with selected beneficial components and restriction of detrimental aliments to modulate gut microbiota to treat or prevent diseases, included IBD and tumors [80].

The most intuitive way to modulate gut microbes is through antibiotics, which are not only imputable of causing dysbiosis. The clearest example is rifaximin, a low toxicity rifamycin derivative poorly absorbable antibiotic

with strong antibacterial effect both on aerobic and anaerobic gram-positive and gram-negative bacteria [81]. Despite its antibiotic properties, rifaximin has also an *eu-biotic* function: indeed, it can promote the growth of favorable bacteria, such as bifidobacteria and lactobacilli, without altering microbial diversity [82]. Moreover, rifaximin seems to have an anti-inflammatory activity, as assessed by the reduction in interleukin-8 and matrix metalloproteinase-9, and its cyclic use is of benefit in treating of symptomatic uncomplicated diverticular disease [83]. This drug is also efficient in preventing cirrhosis complications, such as spontaneous bacterial peritonitis and hepatic encephalopathy recurrence, and its use is associated to reduced endotoxemia and to a functional change of microbial metabolic profile [84]. Several studies reported the effectiveness of rifaximin in patients with IBS and small intestinal bacterial overgrowth compared to placebo [85]. In IBD, there is evidence of effective systemic antibiotic use in disease complications, such as septic ones and fistulas or in chronic pouchitis, but data are still lacking about microbiota-targeted therapy in different phase of activity, which may have a complementary role in future management [86]. Recent findings indicate that antibiotics may be a possible future treatment also in autoimmune diseases, as preclinical models show that broad-spectrum antibiotics can increase T-regs in the intestinal lamina propria and in extraintestinal lymphoid tissues, decrease effector T cells and inflammatory cytokines together with microbial diversity alterations [87].

Administration of probiotics, which are “live microorganisms that when administered in adequate amounts confer a health benefit on the host,” [88] has been studied as prevention strategy or treatment of different diseases. For instances, *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii* may be beneficial in preventing diarrhea caused by dysbiosis associated to antibiotic therapies and *Clostridium difficile* infection [89, 90]. The use of probiotics has been applied in the field of IBD too; indeed, *E. coli* Nissle 1917 and VSL#3 (a mixture of 8 probiotics strains) are frequently prescribed in clinical practice to manage mild-to-moderately active disease flare, mostly in UC [91, 92]. Some authors reported an improvement in specific scores in anxious patients by administering probiotics (mainly lactobacilli), but studies used different measurements methods, strains, dosages, and treatment periods with lack of standardization [93]. A systematic review evaluated 13 randomized controlled trials about probiotic administration compared to placebo in preventing acute upper respiratory tract infections and assessed a significant reduction of the infection rate, of the infectious epi-

sode length, of the necessity to use antibiotics, and of the cold-related school absence, even with a low quality of evidence [94]. Supplementation with lactobacilli seems also effective in preclinical colorectal cancer model to prevent precursor lesions, tumor onset, and growth [95]. Many other studies have been conducted in various clinical conditions, such as metabolic disease, IBS, chemotherapy-induced diarrhea, urinary tract infections, and liver encephalopathy, but evidence is poor [96], and understanding if the efficacy of probiotics is strain-specific and disease-specific is still a major challenge in clinical trials [90].

Gut microbial modulation is also possible by administering prebiotics, which are defined as nondigestible compounds that, through their metabolization by microorganisms in the gut, modulate composition and/or activity of the gut microbiota, thus conferring a beneficial physiologic effect on the host [97]. These compounds are mainly from subset of carbohydrate groups and include fructans, galacto-oligosaccharides, lactulose, starch, and glucose-derived oligosaccharides, as well as noncarbohydrate oligosaccharides [98, 99], which can be fermented by gut bacteria and enhance production of short-chain fatty acids, immunomodulation, and improvement of barrier functions [100]. They might be used alone or in synergic association with probiotics, as synbiotics [101]. Prebiotics may be of benefit in acute gastroenteritis via contrasting pathogens by blocking their adhesion to bowel mucosa, displacing them, or reducing their virulence [102]. Currently, lactulose is part of standard care in treatment of hepatic encephalopathy, as it can reduce the production of toxic ammonia due to its urease activity inhibiting function and its capability to improve nitrogenous product assimilation by gut microbes [103]. The use of prebiotics has been studied in IBD: notably, inulin has showed positive effects due to its capability of promoting the growth of lactobacilli and bifidobacteria and of inducing the production of short-chain fatty acids, with evidence of improved mucosal lesion scores and diminished mucosal inflammation in preclinical models [100]. Evidence of inulin in humans with IBD is limited, but inulin seems promising in reducing endoscopical and histological mucosal inflammation of the pouch after colectomy [104]. The use of prebiotics in IBS has been assessed in some randomized clinical trials, but, even if an amelioration of symptoms occurred, results were almost not significant or affected by biases [105]. Some selected dietary fibers seem to be able to stimulate a butyrate producing colonic ecosystem capable of preventing colorectal cancer by diminishing the rate of aberrant crypt foci formation in mice [106]. Further research will allow us to understand

the potential role of prebiotics and synbiotics in other situations, as immunomodulation, obesity, cognitive defects, metabolic, and cardiovascular diseases [107].

Lastly, an emerging strategy that determines a hard reset of a disturbed microbial ecosystem is fecal microbiota transplantation (FMT), which consists of infusion of fecal material from a healthy donor into the gastrointestinal tract of a patient to heal a specific disease in a context of dysbiosis [108]. This strategy was used empirically in the 4th century in China to cure food poisoning and diarrhea [109] and was reintroduced in modern times in 1958, when Eiseman et al. [110] in Colorado performed fecal enemas to heal few patients with pseudomembranous colitis. Currently, the main worldwide accepted indication to FMT is multiply recurrent *Clostridium difficile* infection [111, 112], and it has been reported that this procedure can reduce disease complications, such as pseudomembranous colitis, bloodstream infections, length of hospitalization, and increase overall survival [113, 114].

In the setting of IBD, FMT showed some efficacy in inducing remission in patients with UC, but further studies are needed to define standardized procedures and outcome predictors [115]. Similarly, FMT may be beneficial for IBS if performed via colonoscopy instead of upper gastrointestinal tract, with apparently higher efficacy when transplanting donor stools rather than autologous stool [116]. The key factor of these procedures is introducing into the recipient a complete healthy gut microbiota and future perspectives involve metabolic disorders, cardiovascular disease, neuropsychiatric disorders, and malignancies treatment with promising results [117].

Conclusion

One of the first ideas of beneficial effects of bacterial administration derived from Elie Metchnikoff's observation in 1905, who supposed that the longevity of the Bulgarian population was provoked by high lactobacilli di-

etary intake from dairy products [118]. Maybe, the initial use of probiotics in alimentary habits to improve health status was only part of cultural beliefs derived from empirical benefits, as it could have been the assumption of castor oil as a popular remedy for constipation [119]. Nowadays, thanks to the advent of new methods to study micro-organisms and to the possibility to perform in vivo and clinical studies, we know that a healthy gut microbiota is crucial for health and that perturbations of its equilibrium are associated with many intestinal and systemic diseases, even if many pathophysiological aspects remain still unknown. Restoring eubiosis, understanding how to modulate gut microbes in disease-specific settings, and elaborating standard protocols of microbiota-driven therapeutic strategies with definite outcomes predictors are the main challenges of researchers of the field. In recent decades, the discovery of gut microbiota could be somehow compared to a modern Pandora's box, a container of secrets that was opened with curiosity and whose flow is hard to stop.

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References

- 1 Smith ER. Epidemiology of gastrointestinal disorders. *Can Fam Physician*. 1978 Oct;24:1007–11.
- 2 Mearin F, Malfertheiner P. Functional Gastrointestinal Disorders: Complex Treatments for Complex Pathophysiological Mechanisms. *Dig Dis*. 2017;35 Suppl 1:1–4.
- 3 Oshima T, Miwa H. Epidemiology of Functional Gastrointestinal Disorders in Japan and in the World. *J Neurogastroenterol Motil*. 2015 Jul;21(3):320–9.
- 4 Knowles SR, Graff LA, Wilding H, Hewitt C, Keefer L, Mikocka-Walus A. Quality of Life in Inflammatory Bowel Disease: A Systematic Review and Meta-analyses-Part I. *Inflamm Bowel Dis*. 2018 Mar;24(4):742–51.
- 5 Talley NJ, Goodsall T, Potter M. Functional dyspepsia. *Aust Prescr*. 2017 Dec;40(6):209–13.
- 6 Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. *Clin Epidemiol*. 2014 Feb;6:71–80.
- 7 Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2018 Dec;390(10114):2769–78.

- 8 Ghosh S. Multidisciplinary teams as standard of care in inflammatory bowel disease. *Can J Gastroenterol*. 2013 Apr;27(4):198.
- 9 Beinvogl B, Burch E, Snyder J, Schechter N, Hale A, Okazaki Y, et al. Multidisciplinary Treatment Reduces Pain and Increases Function in Children With Functional Gastrointestinal Disorders. *Clin Gastroenterol Hepatol*. 2019 Apr;17(5):994–6.
- 10 Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. *World J Gastroenterol*. 2014 Jan;20(1):91–9.
- 11 Holtmann G, Shah A, Morrison M. Pathophysiology of Functional Gastrointestinal Disorders: A Holistic Overview. *Dig Dis*. 2017;35 Suppl 1:5–13.
- 12 Weissshof R, El Jurdi K, Zmeter N, Rubin DT. Emerging Therapies for Inflammatory Bowel Disease. *Adv Ther*. 2018 Nov;35(11):1746–62.
- 13 Whitfield KL, Shulman RJ. Treatment options for functional gastrointestinal disorders: from empiric to complementary approaches. *Pediatr Ann*. 2009 May;38(5):288–90.
- 14 Thabane M, Marshall JK. Post-infectious irritable bowel syndrome. *World J Gastroenterol*. 2009 Aug;15(29):3591–6.
- 15 Navaneethan U. Infections in flares of inflammatory bowel disease. *Am J Gastroenterol*. 2009 Oct;104(10):2634–5.
- 16 Simrén M. Manipulating the Gut Microbiome as a Treatment Strategy for Functional Gastrointestinal Disorders. *Gastroenterology*. 2018 Oct;155(4):960–2.
- 17 Qiao YQ, Cai CW, Ran ZH. Therapeutic modulation of gut microbiota in inflammatory bowel disease: more questions to be answered. *J Dig Dis*. 2016 Dec;17(12):800–10.
- 18 Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JL. Host-bacterial mutualism in the human intestine. *Science*. 2005 Mar;307(5717):1915–20.
- 19 Barghoorn ES, Schopf JW. Microorganisms three billion years old from the precambrian of South Africa. *Science*. 1966 May;152(3723):758–63.
- 20 Salvucci E. The human-microbiome super-organism and its modulation to restore health. *Int J Food Sci Nutr*. 2019 Nov;70(7):781–95.
- 21 Kho ZY, Lal SK. The Human Gut Microbiome - A Potential Controller of Wellness and Disease. *Front Microbiol*. 2018 Aug;9:1835.
- 22 Porter JR. Antony van Leeuwenhoek: tercentenary of his discovery of bacteria. *Bacteriol Rev*. 1976 Jun;40(2):260–9.
- 23 Hematian A, Sadeghifard N, Mohebi R, Taherikalani M, Nasrolahi A, Amraei M, et al. Traditional and Modern Cell Culture in Virus Diagnosis. *Osong Public Health Res Perspect*. 2016 Apr;7(2):77–82.
- 24 Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. *Clin Microbiol Rev*. 2015 Jan;28(1):208–36.
- 25 Rana SV, Malik A. Hydrogen breath tests in gastrointestinal diseases. *Indian J Clin Biochem*. 2014 Oct;29(4):398–405.
- 26 Hiergeist A, Gläsner J, Reischl U, Gessner A. Analyses of Intestinal Microbiota: culture versus Sequencing. *ILAR J*. 2015;56(2):228–40.
- 27 Sarangi AN, Goel A, Aggarwal R. Methods for Studying Gut Microbiota: A Primer for Physicians. *J Clin Exp Hepatol*. 2019 Jan-Feb;9(1):62–73.
- 28 Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol Rev*. 2010 Jul;90(3):859–904.
- 29 Fraher MH, O'Toole PW, Quigley EM. Techniques used to characterize the gut microbiota: a guide for the clinician. *Nat Rev Gastroenterol Hepatol*. 2012 Mar;9(6):312–22.
- 30 Fricker AM, Podlesny D, Fricke WF. What is new and relevant for sequencing-based microbiome research? A mini-review. *J Adv Res*. 2019 Mar;19:105–12.
- 31 Malla MA, Dubey A, Kumar A, Yadav S, Hashem A, Abd Allah EF. Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment. *Front Immunol*. 2019 Jan;9:2868.
- 32 Besser J, Carleton HA, Gerner-Smidt P, Lindsey RL, Trees E. Next-generation sequencing technologies and their application to the study and control of bacterial infections. *Clin Microbiol Infect*. 2018 Apr;24(4):335–41.
- 33 Peterson J, Garg S, Giovanni M, McInnes P, Wang L, Schloss JA, et al. NIH HMP Working Group. The NIH Human Microbiome Project. *Genome Res*. 2009 Dec;19(12):2317–23.
- 34 Bashardes S, Zilberman-Schapira G, Elina E. Use of Metatranscriptomics in Microbiome Research. *Bioinform Biol Insights*. 2016 Apr;10:19–25.
- 35 Kleiner M. Metaproteomics: Much More than Measuring Gene Expression in Microbial Communities. *mSystems*. 2019 May;4(3):e00115–9.
- 36 Fiehn O. Metabolomics—the link between genotypes and phenotypes. *Plant Mol Biol*. 2002 Jan;48(1-2):155–71.
- 37 Smirnov KS, Maier TV, Walker A, Heinzmann SS, Forcisi S, Martinez I, et al. Challenges of metabolomics in human gut microbiota research. *Int J Med Microbiol*. 2016 Aug;306(5):266–79.
- 38 Teimoorian F, Ranaei M, Hajian Tilaki K, Shokri Shirvani J, Vosough Z. Association of Helicobacter pylori Infection With Colon Cancer and Adenomatous Polyps. *Iran J Pathol*. 2018;13(3):325–32.
- 39 Tsay FW, Hsu PI. H. pylori infection and extra-gastrointestinal diseases. *J Biomed Sci*. 2018 Aug;25(1):65.
- 40 Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol*. 2016 Aug;14(8):e1002533.
- 41 Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JP, et al. Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol*. 2009 Oct;11(10):2574–84.
- 42 Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. *Microb Ecol Health Dis*. 2015 Feb;26:26191.
- 43 Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology*. 2014 May;146(6):1489–99.
- 44 Henson MA, Phalak P. Microbiota dysbiosis in inflammatory bowel diseases: in silico investigation of the oxygen hypothesis. *BMC Syst Biol*. 2017 Dec;11(1):145.
- 45 Bamola VD, Ghosh A, Kapardar RK, Lal B, Cheema S, Sarma P, et al. Gut microbial diversity in health and disease: experience of healthy Indian subjects, and colon carcinoma and inflammatory bowel disease patients. *Microb Ecol Health Dis*. 2017 May;28(1):1322447.
- 46 Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA*. 2007 Aug;104(34):13780–5.
- 47 Strauss J, Kaplan GG, Beck PL, Rioux K, Panaccione R, Devinney R, et al. Invasive potential of gut mucosa-derived Fusobacterium nucleatum positively correlates with IBD status of the host. *Inflamm Bowel Dis*. 2011 Sep;17(9):1971–8.
- 48 Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The treatment-naïve microbiome in new-onset Crohn's disease. *Cell Host Microbe*. 2014 Mar;15(3):382–92.
- 49 Bajer L, Kverka M, Kostovcik M, Macinga P, Dvorak J, Stehlikova Z, et al. Distinct gut microbiota profiles in patients with primary sclerosing cholangitis and ulcerative colitis. *World J Gastroenterol*. 2017 Jul;23(25):4548–58.
- 50 Sun Y, Li L, Xia Y, Li W, Wang K, Wang L, et al. The gut microbiota heterogeneity and assembly changes associated with the IBD. *Sci Rep*. 2019 Jan;9(1):440.
- 51 Kassinen A, Kroggius-Kurikka L, Mäkituokko H, Rinttilä T, Paulin L, Corander J, et al. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology*. 2007 Jul;133(1):24–33.
- 52 Chong PP, Chin VK, Looi CY, Wong WF, Madhavan P, Yong VC. The Microbiome and Irritable Bowel Syndrome - A Review on the Pathophysiology, Current Research and Future Therapy. *Front Microbiol*. 2019 Jun;10:1136.
- 53 Helmink BA, Khan MA, Hermann A, Gopalakrishnan V, Wargo JA. The microbiome, cancer, and cancer therapy. *Nat Med*. 2019 Mar;25(3):377–88.
- 54 Vivarelli S, Salemi R, Candido S, Falzone L, Santagati M, Stefani S, et al. Gut Microbiota and Cancer: From Pathogenesis to Therapy. *Cancers (Basel)*. 2019 Jan;11(1):11.

- 55 Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, et al. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity*. 2015 Feb; 42(2):344–55.
- 56 Dalmaso G, Cougnoux A, Delmas J, Darfeuille-Michaud A, Bonnet R. The bacterial genotoxin colibactin promotes colon tumor growth by modifying the tumor microenvironment. *Gut Microbes*. 2014;5(5):675–80.
- 57 Shamriz O, Mizrahi H, Werbner M, Shoenfeld Y, Avni O, Koren O. Microbiota at the crossroads of autoimmunity. *Autoimmun Rev*. 2016 Sep;15(9):859–69.
- 58 Rinaldi M, Perricone R, Blank M, Perricone C, Shoenfeld Y. Anti-Saccharomyces cerevisiae autoantibodies in autoimmune diseases: from bread baking to autoimmunity. *Clin Rev Allergy Immunol*. 2013 Oct;45(2):152–61.
- 59 Vaahntovu J, Munukka E, Korkeamäki M, Luukkainen R, Toivanen P. Fecal Microbiota in Early Rheumatoid Arthritis. *J Rheumatol*. 2008;35:1500–5.
- 60 Luo XM, Edwards MR, Mu Q, Yu Y, Vieson MD, Reilly CM, et al. Gut Microbiota in Human Systemic Lupus Erythematosus and a Mouse Model of Lupus. *Appl Environ Microbiol*. 2018 Jan;84(4):e02288–17.
- 61 van der Meulen TA, Harmsen HJ, Vila AV, Kurilshikov A, Liefers SC, Zhernakova A, et al. Shared gut, but distinct oral microbiota composition in primary Sjögren's syndrome and systemic lupus erythematosus. *J Autoimmun*. 2019 Feb;97:77–87.
- 62 Siljander H, Honkanen J, Knip M. Microbiome and type 1 diabetes. *EBioMedicine*. 2019 Aug;46:512–21.
- 63 Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One*. 2010 Feb;5(2):e9085.
- 64 Pascale A, Marchesi N, Govoni S, Coppola A, Gazzaruso C. The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases. *Curr Opin Pharmacol*. 2019 Dec;49:1–5.
- 65 Jie Z, Xia H, Zhong SL, Feng Q, Li S, Liang S, et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun*. 2017 Oct;8(1):845.
- 66 Karlsson FH, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, et al. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nat Commun*. 2012; 3(1):1245.
- 67 Jin M, Qian Z, Yin J, Xu W, Zhou X. The role of intestinal microbiota in cardiovascular disease. *J Cell Mol Med*. 2019 Apr;23(4):2343–50.
- 68 Janeiro MH, Ramírez MJ, Milagro FI, Martínez JA, Solas M. Implication of Trimethylamine N-Oxide (TMAO) in Disease: Potential Biomarker or New Therapeutic Target. *Nutrients*. 2018 Oct;10(10):1398.
- 69 Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. *J Physiol*. 2017 Jan;595(2):489–503.
- 70 Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord*. 2015 Mar;30(3): 350–8.
- 71 Wasilewska J, Klukowski M. Gastrointestinal symptoms and autism spectrum disorder: links and risks - a possible new overlap syndrome. *Pediatric Health Med Ther*. 2015 Sep; 6:153–66.
- 72 Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, et al. Reduced incidence of *Prevotella* and other fermenters in intestinal microflora of autistic children. *PLoS One*. 2013 Jul;8(7):e68322.
- 73 Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism—comparisons to typical children and correlation with autism severity. *BMC Gastroenterol*. 2011 Mar;11(1):22.
- 74 Cheung SG, Goldenthal AR, Uhlemann AC, Mann JJ, Miller JM, Sublette ME. Systematic Review of Gut Microbiota and Major Depression. *Front Psychiatry*. 2019 Feb;10: 34.
- 75 Gagliardi A, Totino V, Cacciotti F, Iebba V, Neroni B, Bonfiglio G, et al. Rebuilding the Gut Microbiota Ecosystem. *Int J Environ Res Public Health*. 2018 Aug;15(8):1679.
- 76 Muralidharan J, Galie S, Hernandez-Alonso P, Bulló M, Salas-Salvadó J. Plant-Based Fat, Dietary Patterns Rich in Vegetable Fat and Gut Microbiota Modulation. *Front Nutr*. 2019 Oct;6:157.
- 77 Hills RD Jr, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut Microbiome: Profound Implications for Diet and Disease. *Nutrients*. 2019 Jul;11(7):11.
- 78 Panebianco C, Adamberg K, Adamberg S, Saracino C, Jaagura M, Kolk K, et al. Engineered Resistant-Starch (ERS) Diet Shapes Colon Microbiota Profile in Parallel with the Retardation of Tumor Growth in In Vitro and In Vivo Pancreatic Cancer Models. *Nutrients*. 2017 Mar;9(4):331.
- 79 Rangan P, Choi I, Wei M, Navarrete G, Guen E, Brandhorst S, et al. Fasting-Mimicking Diet Modulates Microbiota and Promotes Intestinal Regeneration to Reduce Inflammatory Bowel Disease Pathology. *Cell Rep*. 2019 Mar;26(10):2704–2719.e6.
- 80 Lee D, Albenberg L, Compher C, Baldassano R, Piccoli D, Lewis JD, et al. Diet in the pathogenesis and treatment of inflammatory bowel diseases. *Gastroenterology*. 2015 May;148(6): 1087–106.
- 81 Marchese A, Salerno A, Pesce A, Debbia EA, Schito GC. In vitro activity of rifaximin, metronidazole and vancomycin against *Clostridium difficile* and the rate of selection of spontaneously resistant mutants against representative anaerobic and aerobic bacteria, including ammonia-producing species. *Chemotherapy*. 2000 Jul-Aug;46(4):253–66.
- 82 Ponziani FR, Scaldaferri F, Petito V, Paroni Sterbini F, Pecere S, Lopetuso LR, et al. The Role of Antibiotics in Gut Microbiota Modulation: The Eubiotic Effects of Rifaximin. *Dig Dis*. 2016;34(3):269–78.
- 83 Cuomo R, Barbara G, Annibale B. Rifaximin and diverticular disease: Position paper of the Italian Society of Gastroenterology (SIGE). *Dig Liver Dis*. 2017 Jun;49(6):595–603.
- 84 Ponziani FR, Gerardi V, Pecere S, D'Aversa F, Lopetuso L, Zocco MA, et al. Effect of rifaximin on gut microbiota composition in advanced liver disease and its complications. *World J Gastroenterol*. 2015 Nov;21(43): 12322–33.
- 85 Lee HJ, Choi JK, Ryu HS, Choi CH, Kang EH, Park KS, et al. Therapeutic Modulation of Gut Microbiota in Functional Bowel Disorders. *J Neurogastroenterol Motil*. 2017 Jan;23(1):9–19.
- 86 Ledder O. Antibiotics in inflammatory bowel diseases: do we know what we're doing? *Transl Pediatr*. 2019 Jan;8(1):42–55.
- 87 Nakamura YK, Metea C, Karstens L, Asquith M, Gruner H, Mosciro C, et al. Gut Microbial Alterations Associated With Protection From Autoimmune Uveitis. *Invest Ophthalmol Vis Sci*. 2016 Jul;57(8):3747–58.
- 88 Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014 Aug;11(8):506–14.
- 89 Blaabjerg S, Artzi DM, Aabenhøj R. Probiotics for the Prevention of Antibiotic-Associated Diarrhea in Outpatients: A Systematic Review and Meta-Analysis. *Antibiotics (Basel)*. 2017 Oct;6(4):21.
- 90 McFarland LV, Evans CT, Goldstein EJ. Strain-Specificity and Disease-Specificity of Probiotic Efficacy: A Systematic Review and Meta-Analysis. *Front Med (Lausanne)*. 2018 May;5:124.
- 91 Jia K, Tong X, Wang R, Song X. The clinical effects of probiotics for inflammatory bowel disease: A meta-analysis. *Medicine (Baltimore)*. 2018 Dec;97(51):e13792–13792.
- 92 Ganji-Arjenaki M, Rafieian-Kopaei M. Probiotics are a good choice in remission of inflammatory bowel diseases: A meta analysis and systematic review. *J Cell Physiol*. 2018 Mar; 233(3):2091–103.
- 93 Liu B, He Y, Wang M, Liu J, Ju Y, Zhang Y, et al. Efficacy of probiotics on anxiety-A meta-analysis of randomized controlled trials. *Depress Anxiety*. 2018 Oct;35(10):935–45.
- 94 Hao Q, Dong BR, Wu T. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev*. 2015 Feb;CD006895(2):CD006895.
- 95 Settanni CR, Quaranta G, Bibbò S, Gasbarrini A, Cammarota G, Ianiro G. Oral supplementation with lactobacilli to prevent colorectal cancer in preclinical models. *Minerva Gastroenterol Dietol*. 2019, Epub ahead of print.

- 96 Rondanelli M, Faliva MA, Perna S, Giacosa A, Peroni G, Castellazzi AM. Using probiotics in clinical practice: where are we now? A review of existing meta-analyses. *Gut Microbes*. 2017 Nov;8(6):521–43.
- 97 Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol*. 2015 May;12(5):303–10.
- 98 Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, et al. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. *Foods*. 2019 Mar;8(3):92.
- 99 Bothe MK, Maathuis AJ, Bellmann S, van der Vossen JM, Berressem D, Koehler A, et al. Dose-Dependent Prebiotic Effect of Lactulose in a Computer-Controlled In Vitro Model of the Human Large Intestine. *Nutrients*. 2017 Jul;9(7):767.
- 100 Akram W, Garud N, Joshi R. Role of inulin as prebiotics on inflammatory bowel disease. *Drug Discov Ther*. 2019;13(1):1–8.
- 101 de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. *Adv Biochem Eng Biotechnol*. 2008;111:1–66.
- 102 Joshi D, Roy S, Banerjee S. Chapter 19 - Prebiotics: A Functional Food in Health and Disease. In: Elsevier, 2018. pp. 507–23.
- 103 Sekhar MS, Unnikrishnan MK, Rodrigues GS, Mukhopadhyay C. Synbiotic formulation of probiotic and lactulose combination for hepatic encephalopathy treatment: a realistic hope? *Med Hypotheses*. 2013 Aug;81(2):167–8.
- 104 Welters CF, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG. Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis. *Dis Colon Rectum*. 2002 May;45(5):621–7.
- 105 Ford AC, Harris LA, Lacy BE, Quigley EM, Moayyedi P. Systematic review with meta-analysis: the efficacy of prebiotics, probiotics, synbiotics and antibiotics in irritable bowel syndrome. *Aliment Pharmacol Ther*. 2018 Nov;48(10):1044–60.
- 106 Perrin P, Pierre F, Patry Y, Champ M, Berreux M, Pradal G, et al. Only fibres promoting a stable butyrate producing colonic ecosystem decrease the rate of aberrant crypt foci in rats. *Gut*. 2001 Jan;48(1):53–61.
- 107 Gulzar N, Saleem IM, Rafiq S, Nadeem M. Therapeutic Potential of Probiotics and Prebiotics. Available from: <https://www.intechopen.com/online-first/therapeutic-potential-of-probiotics-and-prebiotics>.
- 108 Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al.; European FMT Working Group. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut*. 2017 Apr;66(4):569–80.
- 109 Zhang F, Luo W, Shi Y, Fan Z, Ji G. Should we standardize the 1,700-year-old fecal microbiota transplantation? *Am J Gastroenterol*. 2012 Nov;107(11):1755.
- 110 Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery*. 1958 Nov;44(5):854–9.
- 111 Cammarota G, Ianiro G, Kelly CR, Mullish BH, Allegretti JR, Kassam Z, et al. International consensus conference on stool banking for faecal microbiota transplantation in clinical practice. *Gut*. 2019 Dec;68(12):2111–21.
- 112 Ianiro G, Valerio L, Masucci L, Pecere S, Bibbò S, Quaranta G, et al. Predictors of failure after single faecal microbiota transplantation in patients with recurrent *Clostridium difficile* infection: results from a 3-year, single-centre cohort study. *Clin Microbiol Infect*. 2017 May;23(5):337.e1–3.
- 113 Ianiro G, Murri R, Sciumè GD, Impagnatiello M, Masucci L, Ford AC, et al. Incidence of Bloodstream Infections, Length of Hospital Stay, and Survival in Patients With Recurrent *Clostridioides difficile* Infection Treated With Fecal Microbiota Transplantation or Antibiotics: A Prospective Cohort Study. *Ann Intern Med*. 2019 Nov;171(10):695–702.
- 114 Ianiro G, Masucci L, Quaranta G, Simonelli C, Lopetuso LR, Sanguinetti M, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy plus vancomycin for the treatment of severe refractory *Clostridium difficile* infection-single versus multiple infusions. *Aliment Pharmacol Ther*. 2018 Jul;48(2):152–9.
- 115 Costello SP, Soo W, Bryant RV, Jairath V, Hart AL, Andrews JM. Systematic review with meta-analysis: faecal microbiota transplantation for the induction of remission for active ulcerative colitis. *Aliment Pharmacol Ther*. 2017 Aug;46(3):213–24.
- 116 Ianiro G, Eusebi LH, Black CJ, Gasbarrini A, Cammarota G, Ford AC. Systematic review with meta-analysis: efficacy of faecal microbiota transplantation for the treatment of irritable bowel syndrome. *Aliment Pharmacol Ther*. 2019 Aug;50(3):240–8.
- 117 Wortelboer K, Nieuwdorp M, Herrema H. Fecal microbiota transplantation beyond *Clostridioides difficile* infections. *EBioMedicine*. 2019 Jun;44:716–29.
- 118 Mackowiak PA. Recycling metchnikoff: probiotics, the intestinal microbiome and the quest for long life. *Front Public Health*. 2013 Nov 13;1:52.
- 119 Paré P, Bridges R, Champion MC, Ganguli SC, Gray JR, Irvine EJ, et al. Recommendations on chronic constipation (including constipation associated with irritable bowel syndrome) treatment. *Can J Gastroenterol*. 2007 Apr;21 Suppl B:3B–22B.