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Minireview

MINIREVIEW

Gut bacteria formation and influencing factors

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One sentence summary: Gut microbiota dynamically changes in response to many internal and external factors, which is closely associated with human diseases.

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ABSTRACT

The gut microbiota plays an important role in human health. In modern life, with the improvement of living conditions, the intake of high-sugar and high-fat diets as well as the large-scale use of antibacterial drugs have an extensive impact on the gut microbiota, even leading to gut microbiota-orchestrating disorders. This review discusses the effects of various factors, including geographic location, age, diet, antibacterial drugs, psychological situation and exercise on gut bacteria, which helps us profoundly to understand the significance of gut bacteria to human health and to find effective solutions to prevent or treat related diseases.

Keywords: gut bacteria; geographic location; age; diet; antibacterial drugs; exercise

ABBREVIATIONS

FMT:	fecal microbiota transplantation;
CS:	caesarean section;
EBF:	exclusively breastfed;
HMP:	Human Microbiota Project;
OTUs:	operational taxonomic units;
WG:	whole grains;
RGs:	refined grains;
DF:	dietary fiber;
SCFAs$^+$:	short-chain fatty acids;
ICIs:	Immune Checkpoint Inhibitors;
T1D:	type 1 diabetes;
T2D:	type 2 diabetes;
PD-1:	programmed cell death protein 1;
PD-L1:	programmed cell death protein ligand 1;
JAX:	Jackson Laboratory (JAX);
TAC:	Taconic Farms;
mAbs$^+$:	monoclonal antibodies;
PIs:	proton pump inhibitors;
AAPs:	atypical antipsychotics;
HPA:	hypothalamic-pituitary-adrenal;

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CNS: central nervous system;
IBD: inflammatory bowel disease;
IBS: irritable bowel syndrome;
IL-1β: leukocyte-1β;
IDO1: indoleamine 2,3 dioxygenase;
IL-23: interleukin-23;
MI: myocardial infarction;
CVD: cardiovascular diseases;
CD: Crohn disease;
UC: Ulcerative Colitis;
rRNA: ribosomal RNA;
CDI: Clostridium difficile infection

INTRODUCTION

The intestine is the main site for microbiota colonization in the body. Typically, when children grow to 3 years old, their gut microbiota compositions are close to the adult's, being a relative stable gastrointestinal microbiota with more than 500 different microbial species (Tang, Li and Hazen 2019). In contemporary world, the economic, social, technological progresses have transformed human lifestyles, and most people in the developed countries has enriched material life. Interestingly, the gut microbiota has also changed and these changes directly or indirectly relate to human multifactorial diseases (Levy *et al.* 2017), such as obesity (Maruvada *et al.* 2017), diabetes (Brunkwall and Orho-Melander 2017), neurodegeneration (Dinan and Cryan 2017), allergy (McKenzie *et al.* 2017) and asthma (Guilleminault *et al.* 2017; Levy *et al.* 2017; Maruvada *et al.* 2017). In Africa, the adoption of western lifestyle and dietary habits also exacerbates the risk of noncommunicable diseases, such as cancer, cardiovascular disease (CVD), and type 2 diabetes (T2D) (Brewster *et al.* 2019). However, the role of the gut microbiota to these diseases remains unclear. But it seems clear that the extensive use of antibiotics has led to colonization resistance loss and antibiotics-induced dysbiosis of intestinal microbiota (Kim, Covington and Pamer 2017). When the gut microbiota is dysfunctional, the proportion of beneficial bacteria is reduced with the increased proportion of harmful bacteria. This imbalance may affect human health in various aspects, including the production of harmful substances such as endotoxin and ammonia, abnormal functions of intestinal peristalsis and hypoimmunity. Dealing with these health issues, there is increasing evidence that fecal microbiota transplantation (FMT) is a promising way to restore a normal microbiota and thus cure the disease. It is worthy to mention that FMT is still a black box with unknown risks and side effects, especially some particular metabolites or drugs in the feces that adversely affect the recipient patients. For instance, valerate is a metabolite that may be a critical determinant in treating recurrent *Clostridioides difficile* infection (CDI) via FMT, but its minimum dose and mechanism have not been determined (Cresci and Bawden 2015; Bibbo *et al.* 2017; Vindigni and Surawicz 2017; McDonald *et al.* 2018; Mullish *et al.* 2018). Although the relationship between gut microbes and diseases has drawn great attention, many issues are yet to be addressed. These include how to identify beneficial bacterial species and select potential immunomodulatory metabolites from dietary constituents (Wypych, Marsland and Ubags 2017), and whether there is a causal relationship between dysbiosis of gut flora and occurrence of autoimmune diseases (Bach 2018).

The gastrointestinal tract is a major site of microbial colonization and growth (Riaz Rajoka *et al.* 2018). Although humans form a stable intestinal flora at the age of 3, this relative stability is affected by the following factors in a different way, including geographic location, age, diet, antibacterial agents, psychological situation, stress and exercise (Cresci and Bawden 2015; Gupta, Cifu and Khanna 2018). Moreover, in response to different influencing factors there are different dominant or low abundance bacteria being observed (Table 1). Although the terms *microbiota*, the bacteria, archaea, protists, fungi and viruses and *microbiome*, the bugs and their genes, are often used synonymously, they in fact represent slightly different entities. Here, we focus our review mainly on bacteria.

FORMATION OF GUT MICROBIOTA

Initially, it is believed that the fetus is sterile in the uterus, and the first colonization of the intestinal flora occurs during the giving-birth process (Salazar *et al.* 2014). This traditional understanding had been revolutionized following the discovery of microorganisms in amniotic fluid, the umbilical cord, placenta and preterm infant meconium (Cresci and Bawden 2015). The process from birth to the formation of adult-like gastrointestinal microbiota is influenced by many determinants, as summarized in Fig. 1.

The human gut serves as an essential habitat for a diverse microbiota ecosystem. At birth, when infants are continuously exposed to the external environment, the intestines are quickly colonized by trillions of microbes (10^{13}–10^{15}) (Tang, Li and Hazen 2018). The early establishment of gut microbiota is important for subsequent health (Rautava 2016; Ho *et al.* 2018), with gut microbiota compositions showing differences by delivery mode (Munyaka, Khafipour and Ghia 2014; Cresci and Bawden 2015; Rutayisire *et al.* 2016). It was manifested that caesarean section (CS) had the higher abundance and diversity of the Phylum *Firmicute* (*Clostridium* and *Lactobacilli*), but at the colonization level, *Bifidobacterium* and *Bacteroides* genera were significantly more frequent in vaginally delivered infants than CS infants (Adlerberth *et al.* 2007; Rutayisire *et al.* 2016). However, this diversity and colonization pattern of the gut microbiota are only significantly associated with the mode of delivery in the first three months of life, and this difference gradually disappears within 6 months after birth.

After birth, the intestinal microbes of an infant are mainly from the mother's skin, breastfeeding and/or formula feeding (Pannaraj *et al.* 2017; Ho *et al.* 2018), and 25%–30% of the bacterial microbiota is derived from breast milk (Pannaraj *et al.* 2017; Robertson *et al.* 2019). Breast milk is considered the golden standard for infants, but it is not sterile and contains about 600 different bacteria species including *Bifidobacterium* species, such as *Bifidobacterium breve*, *Bifidobacterium adolescentis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, and *Bifidobacterium dentium* (Mohammadkhah *et al.* 2018). In breast milk, oligosaccharides have been reported to play an essential role in promoting the colonization of microorganisms and are beneficial to the growth of microorganisms, especially *Bifidobacteria* (Charbonneau *et al.* 2016; Riaz Rajoka *et al.* 2018). Infants exclusively breastfed (EBF) and non-EBF were significantly different in their aspects of intestinal microbial diversity, microbiota age, microbial compositions and functions in the first 6 months after birth (Cresci and Bawden 2015; Pannaraj *et al.* 2017; Ho *et al.* 2018). After 6 months of age, these differences sustain, and EBF infants have a shorter duration of having higher abundance in *Lactobacillaceae*, *Coriobacteriaceae*, *Prevotellaceae*, *Clostridiaceae*, *Erysipelotrichaceae* and *Lachnospiraceae*, and lower abundance in *Bifidobacteriaceae* and *Enterococcaceae* (Ho *et al.* 2018).

Table 1. The relative beneficial or harmful bacteria in the gut microbiota in response to influencing factors.

Influencing factors		Beneficial/dominant bacteria	Harmful/low abundance bacteria	References
Geographic location	Japan	*Bacteroidetes, Firmicutes, Actinobacteria, Bifidobacterium*	*Clostridium, Alistipes*	(Nishijima *et al.* 2016; Mano *et al.* 2018)
	China	*Bacteroidetes, Proteobacteria, Firmicutes, Actinobacteria*	*Megamonas, Succinivibrio, Prevotella*	(Kuang *et al.* 2016; Liao *et al.* 2018)
	India	*Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Prevotella*	/	(Kumbhare *et al.* 2017; Brewster *et al.* 2019; Tandon *et al.* 2018)
	Argentinan	*Ruminococcaceae, Lachnospiraceae, Rikenellaceae, Prevotellaceae*	/	(Carbonetto *et al.* 2016)
	USA	*Ruminococcaceae, Lachnospiraceae, Rikenellaceae Prevotellaceae*	/	(Carbonetto *et al.* 2016; Hansen *et al.* 2019)
Age	Infants	*Bifidobaterium, Fusobacteria, Proteobacteria*	*Enterococcus faecalis, Methanobrevibacter*	(Cresci and Bawden 2015; Milani *et al.* 2017)
	Adults	*Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Cyanobacteria, Verrucomicrobia*	*Bifidobacteria, Clostridium cluster XIVa*	(Ottman *et al.* 2012; Salazar *et al.* 2017)
	The elderly (>65)	*Bacteroidetes, Clostridia, Lactobacilli, Streptococci, Enterobacteriaceae, Proteobacteria, Escherichia coli*	*Firmicutes, Clostridium XIVa, Faecalibacterium prausnitzii, Actinobacteria*	(O'Toole and Jeffery 2015; Salazar *et al.* 2017; An *et al.* 2018)
	Centenarians	*Akkermansia, Clostridium XIVa, Bifidobacterium*	*Faecalibacterium, Roseburia, Coprococcus, Blautia,*	(Biagi *et al.* 2016; Kong *et al.* 2016; Biagi *et al.* 2017; Kong *et al.* 2018)
Diet	Thai vegetarians	*Prevotella copri, Faecalibacterium prausnitzii*	*Alistipes putrednis, Bilophila wadsworthia*	(Ruengsomwong *et al.* 2016)
	Thai non-vegetarians	*Bacteroides vulgatus, Faecalibacterium prausnitzii*	*Bilophila wadsworthia, Coprococcus eutactus*	(Ruengsomwong *et al.* 2016)
	WGs	*Lachnospira, Roseburia*	*Enterobacteriaceae*	(Vanegas *et al.* 2017)
	DF and prebiotic	*Actinomycetes, Bifidobacteriaceae, Lactobacilli*	*Ruminococcus, Lachnobacterium, Anaerostipes, Ruminococcus, Escherichia coli*	(Holscher 2017; Mano *et al.* 2018)
	Mediterranean diet	*Bacteroidetes, Roseburia, Lachnospira, Prevotella, Firmicutes, Candida*	*Escherichia coli*	(De Filippis *et al.* 2016; Mitsou *et al.* 2017; Ni Lochlainn, Bowyer and Steves 2018)
Antibacterial drugs	Antibiotics	*Bacteroidetes, Proteobacteria*	*Bifidobacterium, Akkermansia muciniphila, Lactobacilli*	(Sivan *et al.* 2015; Ianiro, Tilg and Gasbarrini 2016; Routy *et al.* 2018)
Psychological situation and stress	Depression, anxiety	*Enterobacteriaceae, Alistipes, Bacteroidales*	*Faecalibacterium, Lachnospiraceae*	(Jiang *et al.* 2015; Liu 2017)
	Psychological stress and social stress	*Firmicutes, Clostridium*	*Lactobacillus spp, Bifidobacteria, Lactobacilli, Alistipes*	(Liu 2017; Lach *et al.* 2018)
Exercise	Competitive cyclists	*Prevotella, Methanobrevibacter smithii, Bacteroidetes, Firmicutes, Euryarchaeota*	*Achromobacter, Brachymonas, Candidatus, Desulfobulbus, Leptothrix*	(Petersen *et al.* 2017; Allen *et al.* 2018)
	Rugby athletes	*Akkermansia muciniphila*	*Bacteroide, Lactobacillus*	(Petersen *et al.* 2017; Barton *et al.* 2018)
	Elite athletes, rowers	*Veillonella*	-	(Scheiman *et al.* 2019)

In humans at age of 3, the composition of gut microbiota remains relatively stable over time with introduction of solid food (Cresci and Bawden 2015; Liu *et al.* 2016; Ho *et al.* 2018; Mohammadkhah *et al.* 2018). Nevertheless, throughout their life, humans are exposed to a variety of living conditions, especially taking high-fat diets and antibacterial drugs, and as consequences, the gut microbiota may develop into the direction being healthy or detrimental to the body (Salazar *et al.* 2014). Thus, the formation of gut microbiota undergoes a long period from the first establishment in new-born babies to the stability in children, and this relative stability is dynamically fluctuated and even disrupted by external factors.

INFLUENCING ELEMENTS OF HUMAN GUT MICROBIOTA

The gut microbes are increasingly considered to have a fundamental role in human physiology and health (Rothschild *et al.* 2018). Under normal circumstances, intestinal microbes are in equilibrium and coexist with the host in a mutualism way. The human gut provides the habitat and nutrients needed for gut microbiota growth, and the gut microbiota in turn provides substrates, enzymes, vitamins and energy for human metabolic processes (Arumugam *et al.* 2011). Simultaneously, the short chain fatty acids (SCFAs) produced by microbial metabolism

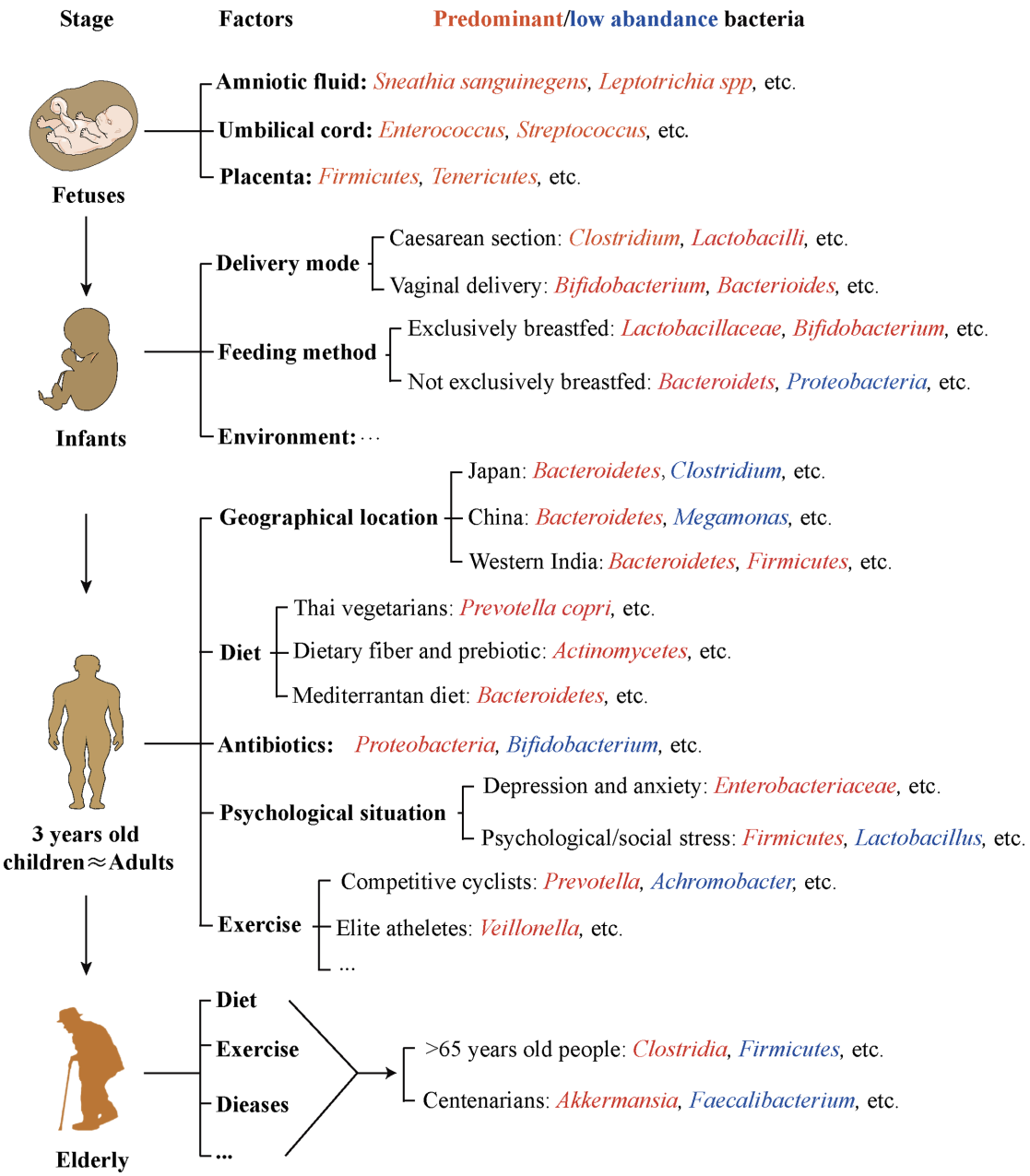

Figure 1. The influencing factors of gut bacteria formation in humans. Different influencing factors are involved in the gut bacteria formation in humans from the fetuses to the elderly. In humans at age of 3, the composition of gut microbiota becomes relatively stable over time and is similar to the adult's. In response to the influencing factors, some bacteria become predominant (in red), but some become lowly abundant (in blue).

promote the growth and differentiation of human epithelial cells and participate in the synthesis of vitamins and the absorption of various ions. The intestine is considered to be the largest immune organ in the body. The communication between the intestinal microbes and the host on the surface of the intestinal mucosa also promotes the establishment and development of the immune system. For example, the robust enterohaemorrhagic *Escherichia coli* colonization in the mammalian intestine relies on the fucose-sensing system to modulate the pathogenicity and virulence gene expression (Pacheco *et al.* 2012). Therefore, the gut microbiota may be one of the standards for characterizing the healthy state of the body (Kong *et al.* 2018).

Geographical location

The geographical location affects the diversity and composition of gut microbiota in infants and adults (Table 1) (Cresci and Bawden 2015). The geographical specificity may imply that it is impossible to have a universal 'healthy microbiota'. A study in different geographical populations supported the idea of the differences in intestinal microbiota compositions in people living in different continents (Magne *et al.* 2016). For example, the Phyla *Bacteroidetes, Firmicutes* and *Actinobacteria* are the main three kinds of gut bacteria in Japanese, partially due to their staple food structure (Mano *et al.* 2018). In China, Yao populations, who are a relatively isolated minority and live in the

heart of the forest, have a unique genus *Megamonas* compared to the local minority Zhuang and rural Han people. Unlike the Japanese, the dominant bacteria of these three groups are the genus *Bacteroides* and *Prevotella* (Liao *et al*. 2018). With the urbanization of China, the higher abundance of microbes in the urban population is the same as Americans', while archaea and viruses are gradually lost compared to the rural population in China (Winglee *et al*. 2017). This phenomenon may be attributed to geographical distribution and the changes in foods and environment. In western India, the gut microbiota in an adult urban population mainly consisted of four phyla, *Bacteroidetes* (71.5%), *Firmicutes* (18.7%), *Proteobacteria* (3.8%) and *Actinobacteria* (0.6%) (Tandon *et al*. 2018). Similarly, Latin Americans are significantly different in gut microbiota from those who are from other continents. For instance, *Firmicutes* are decreased but *Bacteroidetes* have no changes in Colombian adults compared with Americans, Europeans and Asians (Escobar *et al*. 2014; Magne *et al*. 2016). Moreover, *Ruminococcaceae*, *Lachnospiraceae*, *Rikenellaceae* and *Prevotellaceae* are more abundant in Argentinan than Americans (Carbonetto *et al*. 2016; Hansen *et al*. 2019).

To further evaluate the geographical effects, the gut microbiota composition in different origin populations was measured based on β diversity analysis and showed that Europeans were significantly different from Indians (Rehman *et al*. 2016). Even for people who live in geographically adjacent places, their gut microbiota share a common diversity index and similar α diversity (the diversity within single sample), as evidenced by that the observed number of operational taxonomic units (OTUs) is dramatically higher in the rural Bassa infants than urban infants and adults (Ayeni *et al*. 2018). Therefore, geographical location as an influencing factor should be taken into account in the evaluation of the composition and diversity of the gut microbiota.

Age

Due to the introduction of solid foods, the structural and functional diversity of the infant microbiota is rapidly increased (Table 1). Once forming a microbial structure like the adult's, it remains relatively stable until old age (Cresci and Bawden 2015; Robertson *et al*. 2019). In terms of gut microbiota in infants, *Bifidobaterium* resides as the most dominant, and as time goes, the phyla *Firmicutes* and *Bacteroidetes* occupy the dominant part in adults, while *Bacteroidetes* become a relatively predominating proportion in the elderly (generally > 65 years old), with an increase of *Clostridia*, *Lactobacilli*, *Streptococci* and *Enterobacteriaceae* (Ottman *et al*. 2012; Cresci and Bawden 2015; O'Toole and Jeffery 2015; An *et al*. 2018). It is noted that these results are derived from fecal samples mostly representing the end of the colon but not the entire gut. In addition to the factor of age, this differential distribution of gut microbiota may be also attributed to the external factors such as diet and exercise (O'Toole and Jeffery 2015).

The intestinal microbiota consist of various phyla that contain over 1000 different microbial species (Riaz Rajoka *et al*. 2018). Based on the cultivation and classical 16S ribosomal RNA (rRNA) gene approach, α diversity was found to decline during senescence, but this finding was not further determined by other methods, such as high-throughput 16S rRNA gene sequencing and phylogenetic microarray analysis (An *et al*. 2018). Therefore, the complex relationship of microbial changes with age needs to be systematically investigated. In addition, some studies have found that the Alzheimer's type dementia and frailty in the elderly are related to their own fecal microbiota imbalance, and the increased frailty has been shown to be associated with reduced α diversity (Bian *et al*. 2017; An *et al*. 2018). The longevity (> 90 years old) with a healthy state may be associated with a similar composition of fecal microbiota as seen in healthy young people (O'Toole and Jeffery 2015; Ticinesi, Tana and Nouvenne 2019). However, the diversity of the gut microbiota is greater in longevity people than young adults (Kong *et al*. 2018). Centenarians are shown to have the ability to delay or avoid chronic diseases. These may be attributed to some beneficial bacterial taxa (e.g. *Akkermansia* and *Clostridium XIVa*), which are known as SCFA producers enriched in the long-living people in China and Italy (Kong *et al*. 2016; Kong *et al*. 2018). However, the mechanisms by which age changes affect gut microbiota remain unclear, and need to be explored in the broader context of the genetic and lifestyle changes that accompany aging (Fontana and Partridge 2015; O'Toole and Jeffery 2015).

Diet

It is now becoming clear that diet is a major driving force of microbial dynamics (Gibbons *et al*. 2017). The long- and short-term changes in dietary composition are effective in changing the structure and activity of gut microbiota (Table 1) (David *et al*. 2014; Lam, Zhang and Zhao 2018). The dominant flora in the human gut is related to the ratio of microbiota-accessible carbohydrates, dietary fats and proteins in the dietary structure. The restriction of microbiota-accessible carbohydrates leads not only to bacterial diversity loss but also to SCFA reduction, which eventually affect many physiological processes including energy homeostasis, lipid metabolism and inflammation. Total dietary proteins and amino acid compositions are also influencing factors for SCFA production, but the molecular mechanism is still not fully understood (Gentile and Weir 2018). Consistent with this idea, there is a significant difference in the gut microbiota of vegetarians versus non-vegetarians. In the gut microbiota of healthy Thais, the strong species indicator of vegetarians was *Prevotella copri*, while the species indicator of non-vegetarians was *Bacteroides vulgatus* (Ruengsomwong *et al*. 2016). Furthermore, the effects of a diet enriched with whole grains (WGs) versus refined grains (RGs) on gut microbiota are also remarkable. There was a significant decrease in *Enterobacteriaceae* in the WGs group versus the RGs group, but at the genus level, the *Lachnospira* abundance was increased in WGs group compared with the RGs group (Vanegas *et al*. 2017).

In addition, dietary fiber (DF) intake also contributes to the changes of intestinal microbes. SCFAs produced by DF fermentation enhance intestinal barrier functions by increasing intestinal cell proliferation and differentiation (Kieffer, Martin and Adams 2016). People who have a dietary pattern of eating rice as staple food can ingest higher DF-containing food compared to those who take edible wheat flour products as staple food (Batres-Marquez, Jensen and Upton 2009). In a recent study, according to the intake of staple rice and white bread, Japanese subjects were divided into two groups, and then cross-over experiments were conducted to exchange staple food. It was found that the staple food change made *Actinomycetes* and *Bifidobacteriaceae* significantly higher after the bread period than the rice period (Mano *et al*. 2018).

Furthermore, some people have special habits and customs, such as smoking and alcohol consumption that also contribute to the gut microbiome dysbiosis. It has reported that people who smoke have an increased CDI rate compared than those who never smoke, and people who drink alcohol have the depletion of anti-inflammatory bacteria (*Firmicutes*, *Pediococcus*, *Lactobacillus* and *Leuconostoc*), eventually leading to intestinal leakage (Capurso and Lahner 2017).

It is well known that dietary habit varies from one place to another around the world, so it is unlikely that a universal dietary habit or structure that is generally beneficial to the health of the intestine exists. However, there is a fairly popular diet model, the Mediterranean diet, which is considered to be beneficial to gut microbiota and is associated with lower incidence of common chronic diseases (Bowyer *et al*. 2018; Pignanelli *et al*. 2018). This diet is characterized by high intake of whole grains, vegetables, beans, fruits, unsaturated lipids and fish, by low intake of saturated fats, meat and dairy products, and by moderate drinking (Buford 2017; Mitsou *et al*. 2017; Barton *et al*. 2018; Pignanelli *et al*. 2018).

All in all, different diets have different effects on the structure, composition and function of intestinal microbial flora. Indeed, in a highly controlled mouse nutritional study, differences only in starch composition, not starch quantity, gave rise to an altered microbiota composition (Fernandez-Calleja *et al*. 2018). Therefore, the establishment of the plasticity of gut microbes is a promising strategy to prevent and cure related diseases, but it mainly relies on long-term dietary patterns and stable microbiota conformations instead of short-term diet interventions.

Antibiotics and non-antibiotic drugs

Generally, the intestinal microbial flora is relatively stable and robust, and after a small range of disturbances, it will return to normal status unless external factors endure for a long time. High frequent use of drugs like microbiota's nemesis-antibiotics often disrupts the intestinal microbiota balance and destroys the cross-talks between microbiota and immune fitness (Table 1) (Botticelli *et al*. 2017). Moreover, allergic and autoimmune diseases are also largely attributed to the use of antibiotics in early life, such as type 1 or 2 diabetes (T1D or T2D) (Cox *et al*. 2014; Candon *et al*. 2015; Tai, Wong and Wen 2015), asthma (Stokholm *et al*. 2014), CVD, cancer and obesity (Iizumi *et al*. 2017; Yi *et al*. 2018).

Recently, immune checkpoint inhibitors (ICIs) were recognized as a greatly efficient target to improve the survival of patients who suffered from metastatic melanoma, lung carcinoma, renal cell carcinoma and lymphoma (Botticelli *et al*. 2017). This idea has shifted attention from the tumor itself to the patient's immune system and multiple intersecting immune regulatory networks, such as the PD-1/PD-L1 axis (programmed cell death protein 1/programmed cell death protein ligand 1) (Botticelli *et al*. 2017; Routy *et al*. 2018). It has been shown that there are only 20%–30% clinical benefits, and its efficiency will be largely reduced because of the use of antibiotics (Elkrief *et al*. 2018; Soularue *et al*. 2018). PD-1 based immunotherapy is influenced by gut microbiota, such as commensal *Bifidobacterium* (Sivan *et al*. 2015) and *Akkermansia muciniphila* (Routy *et al*. 2018). In genetically similar, but not the same, C57BL/6 mice from Jackson Laboratory (JAX) and Taconic Farms (TAC), oral administration of JAX fecal suspension to TAC mice enhanced spontaneous antitumor immunity (making tumor growth significantly slower), which was consistent with αPD-L1 monoclonal antibody (mAb) therapy and combination therapy of JAX fecal transfer and αPD-L1 mAb. This antitumor effect was mainly attributed to commensal *Bifidobacterium* (Sivan *et al*. 2015). It was also shown that antibiotics decreased the clinical benefit of ICIs in advanced cancer patients, and FMT from cancer patients who responded to ICIs improved the PD-1 blockade antitumor functions. Further analysis demonstrated that oral supplementation with *Akkermansia muciniphila* restored the efficacy of PD-1 blockade in antibiotics-treated mice that did not respond to ICIs. This restoration was in an interleukin-12–dependent manner by increasing the recruitment of $CCR9^{+}CXCR3^{+}CD4^{+}$ T lymphocytes (Routy *et al*. 2018). Therefore, maintaining a healthy gut microbial flora can support patients in their treatment of cancer.

It is also noted that non-antibiotic drugs, such as antidiabetics like metformin (Forslund *et al*. 2017) and proton pump inhibitors (PPIs) (Jackson *et al*. 2016), inhibit the growth of at least one bacterial strain, which in turn regulates the efficacy and toxicity of the drug (Maier *et al*. 2018). In T2D patients receiving metformin treatment, *E. coli* was significantly increased and *Intestinibacter* decreased compared with the untreated group. Consistently, subsequent preclinical experiments showed that the blood glucose levels were reduced in the germ-free FMT mice and the gastrointestinal side effects were mainly caused by an increase of *E. coli* species. Unlike metformin, PPI contributes to the reduction of commensal bacteria diversity, leading to a decreased colonisation resistance to intestinal infections (including *Clostridium difficile*, *Campylobacter* and *Salmonella*) (Weersma, Zhernakova and Fu 2020). In summary, both antibiotics and non-antibiotic drugs affect the gut microbiome via different mechanisms and ultimately influence health outcomes.

Psychological situation and stress

Stress is an overall response to environmental needs or pressures in organisms. Mental stress can cause a series of physiological changes in the gastrointestinal tract, including gut motility, visceral perception, gastrointestinal secretion and intestinal permeability, which lead to activation of the hypothalamic-pituitary-adrenal (HPA) axis, decrease of mucosal immune functions and alteration of the release of duodenal bicarbonate and hormones (Cresci and Bawden 2015). These changes will alter the gut microbial composition through the gut-brain axis, a bidirectional communication between the gut microbiota and the central nervous system (CNS) (Dinan and Cryan 2017b; Bercik, Collins and Verdu 2012; Cresci and Bawden 2015; Tetel *et al*. 2018). Accordingly, early life stress can lead to disability and prevalence of stress-related mental illnesses (such as anxiety and depression) and gastrointestinal diseases (such as inflammatory bowel disease (IBD)) and irritable bowel syndrome (IBS) (de Weerth 2017). Although there is limited evidence to demonstrate that it causes a rising trend of IBD, stress has an effect on the symptom development of IBD (Bernstein 2017). When rodents have the symptoms and behaviors of anxiety, their jejunum showed an increased abundance in *Campylobacter* and *Citrobacter* (Bruce-Keller *et al*. 2015). Similarly, patients suffering from depression also have higher levels of *Enterobacteriaceae* and *Alistipes* compared with healthy adults (Jiang *et al*. 2015). However, it has been demonstrated that *Bifidobacterium breve A-1* has a potential role in improving anxiety and depressive symptoms in patients (Okubo *et al*. 2018). These alterations of the gut microbial composition by psychological and social stress are related to not only changes of cytokines but also an increased level of catecholamines (Table 1) (Foster and McVey Neufeld 2013; de Weerth 2017; Liu 2017).

In turn, the intestinal microbiota also affects the psychological situation, and gut microbial disorders lead to the risk of mental illness, especially depression and anxiety (Liu 2017). It has been shown that the transfer of fecal materials from natural stress-sensitive mice to non-anxiety mice triggers an anxiety-like phenotype. Conversely, the transfer of bacteria from non-anxiety mice to recipients with natural anxiety leads to a

decrease in anxiety (Bercik *et al.* 2011; Sarkar *et al.* 2018). However, there have so far been no reports using fecal transplantation for treating anxiety and depression in humans (Sarkar *et al.* 2018).

Physical exercise

It is well known that physical exercise promotes the metabolism and immunity ability of the human body (Kurilshikov *et al.* 2017; Petersen *et al.* 2017; Allen *et al.* 2018; Song and Chan 2018), though it might also adversely effect intestinal permeability (JanssenDuijghuijsen *et al.* 2016). A recent study showed that physical exercise affect the health of the gut flora by altering the composition of the gut microbiota with an increased proportion of microorganisms that contribute to intestinal health (Okubo *et al.* 2018; Scheiman *et al.* 2019). Physical exercise endurance was shown to be enhanced in mice and elite athletes, being attributable to relatively increased abundance of *Veillonella*. This kind of bacteria contribute to metabolize muscle-derived lactic acid (entering into the colon through the epithelial barrier) to produce the SCFA propionic acid, which improves endurance performance (Scheiman *et al.* 2019). In addition, in intestinal microbes-free mice, the colonization of intestinal microbiota resulted in a significant decrease in the expression of innate inflammatory mediators after transplantation of the stool of the excise mice group (containing the intestinal microbial flora), like leukocyte-1β (IL-1β), indoleamine 2, 3 dioxygenase (IDO1) and interleukin-23 (IL-23). Further analysis revealed that there was a high proportion of specific microorganisms in the intestine (Allen *et al.* 2018), which are responsible for SCFA production, such as acetate, propionate and butyrate that have a beneficial effect on cardiometabolic health and intestinal cell survival (de la Cuesta-Zuluaga *et al.* 2018; Tye *et al.* 2018).

It was also demonstrated that the number of beneficial bacteria was significantly increased in the gut, and the levels of SCFAs were also increased by physical excise, particularly butyrate, a major substrate for colonocytes which represents the symbiosis between host and microbiota (Tye *et al.* 2018). Consistently, compared to healthy controls, gut microbial diversity of these rugby players was increased and enriched in the SCFA synthesis under execise and high protein diet (Barton *et al.* 2018). However, competitive cyclists had a higher abundance of *Prevotella*, which was negatively related to SCFA metabolism (Petersen *et al.* 2017). Moreover, fecal metabolites, including acetate, propionate and butyrate, are relatively increased in professional international rugby union athletes, which are associated with health benefit phenotypes (Barton *et al.* 2018).

In myocardial infarction (MI) mice, moderate intensity exercise was demonstrated to lead to intestinal microbial structural changes associated with cardiac functions, and two kinds of the bacteria *Butyricimonas* and *Akkermansia* were significantly enriched, which might play a beneficial role before the development of CVD (Liu *et al.* 2017). Similarly, it was also found that physical exercise was the most cost-effective lifestyle intervention to prevent and treat diabetes by altering gut microbiota, expecially *Alistipes shahii*, *Alistipes putredinis*, *Ruminococcus gnavus*, *Eubacterium hallii* and *Coprococcus comes* that may be related to the improvements in glucose homeostasis and insulin sensitivity (Liu *et al.* 2020). These findings render the feasibility of disease prevention via expanding the benefits of physical exercise and thus changing the gut microbiota. However, high-intensity exercise likely has a negative impact on gastrointestinal tract and leads to discomfort (Cresci and Bawden 2015), as evidenced by that 30%–90% distance runners have GI problems (de Oliveira, Burini and Jeukendrup 2014; Cresci and Bawden 2015).

CHALLENGES AND PROSPECTS

Gut microbiota play a vital role in human health. The development and use of intestinal flora-related methods and tools, including FMT, enterotype classification and innovations for gut bacterium culture, has broad prospects in the treatment of related diseases in future.

Although it has been clinically used in the treatment of gut microbe-orchestrating diseases, the long-term efficacy of FMT needs further monitoring. One of key issues is no uniform standard for the specific number of fecal bacteria used in FMT, leading to inconsistent clinical treatment results. Another is that FMT donors may have specific risk factors associated with a certain physical condition, such as metabolites, viruses and pathogenic bacteria that are normally difficult to be monitored. Therefore, it is urgent to establish a robust system to evaluate and supervise FMT from preparation to administration.

The simple classification of gut microbiota structures by gut enterotypes has a clinical potential, such as identifying an individual's disease situation and serving as a useful biomarker in disease progression. When we rely solely on gut enterotypes, the important microbial variations may be masked to some extent (Costea *et al.* 2018). It will be of high interest to investigate gut microbiota as a biomarker or index for age change, diet intake and disease progression, and for evaluation of the effects of probiotics, prebiotics and synbiotics (combination with prebiotic fiber) on clinical therapy.

Recently, a great progress has been made in culturing gut microbiota, but 23%–65% of microorganisms colonized in the human intestine cannot be cultured *in vitro*, which becomes an obstacle for exploring their biological functions (Strandwitz *et al.* 2019). The rise of omics analysis provides an opportunity to better understand the intestinal microbial flora. Using high-resolution metagenomic sequencing, the key bacterial species as potential predictors have been identified in some common GI diseases, such as IBD and IBS (Vich Vila *et al.* 2018). However, the omics can not distinguish viable or relic DNA and in many human studies, only feces rather than intestines' digesta is collected. Therefore, exact microbiota dynamics is masked, which results in why most probiotics work well in the laboratory but not in clinical. It should be noted that potentially confounding factors of technique and biology have a mixed effect on the characteristics of intestinal microbiota markers, so further work is needed to solve the issues in exploring the influencing elements on gut microbiota (Sonnenburg and Backhed 2016; Kurilshikov *et al.* 2017; Jackson *et al.* 2018).

Measuring the direct interaction of the gut microbiota with food (components) also attracts more and more attention. Recent developments in this field include for instance usage of high tech telemetric capsules in humans, or non-invasive gut microbiota activity measurements using gas sensors detecting fermentation gasses in exhaled air [Nishijima *et al.* 2016]. The latter approach has been shown, using mouse models, to be able to quantitatively detect hydrogen levels in real-time, and an altered activity pattern within a few hours after switching from chow to a low digestible maize-starch diet, independent of total starch intake [Kuang *et al.* 2016; Fernandez-Calleja *et al.* 2018].

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

AUTHOR CONTRIBUTIONS

YZ conceived the study. JY, JW, YL, YeZ, XJ, WCC, EvS and YZ wrote the paper.

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Conflicts of Interest. None declared.

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