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Review

Food Additives and Contaminants: Effects on Human Gut Microbiota—A Review

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Abstract: Gut bacteria play an important role in several metabolic processes and human diseases, such as obesity and its co-morbidities, like fatty liver disease, insulin resistance/diabetes and cardiovascular events. Among several factors, dietary patterns, probiotics, prebiotics, synbiotics, antimicrobials and non-dietary factors, such as stress, age, exercise and climatic conditions, can dramatically impact the human gut microbiota diversity and equilibrium. However, the effect of minor food constituents, including food additives and trace contaminants, on human gut microbiota has received less attention. Consequently, the present review aimed to provide an objective perspective of the current knowledge regarding the impacts of minor food constituents on human gut microbiota and consequently, on human health.

Keywords: antibiotic; bacteroidetes; dietary emulsifier; firmicutes; food additive; gut microbiota; non-nutritive sweetener; proteobacteria

1. Introduction

Humans have approximately 10 times as many microorganisms within their gastrointestinal tract (GI) (approximately 100 trillion) than the number of somatic cells within their body (10 trillion cells) [1,2]. Consequently, the gut microbiota (GM) plays a major role in health and disease in humans: indeed, it is sometimes referred to as our "forgotten organ" [3].

The GM play an important role in several human diseases, such as obesity [4,5], diabetes [6,7], cancer [8,9], cardiovascular diseases [10] metabolic syndrome [1,3,11] non-alcoholic fatty liver disease [12,13] and in several psychiatric disorders [14,15]. Gut microbes produce a large number of bioactive compounds that can influence human health. Some (such as vitamins) are beneficial, but other products can be toxic [1]. Additionally, the GM interacts with the immune system, providing signals to promote the maturation of immune cells and the normal development of immune functions [16, 17]. In this context, GM microbes contribute to maintaining the integrity of the intestinal epithelium, preserving cell-to-cell junctions, promoting epithelial repair following injury, and playing an important role in the regulation of enterocytes turnover [18].

Since it was reported that compared to Italian children, the fecal microbiota of children from a rural African village of Burkina Faso (high-fiber diets) possessed a unique abundance of bacteria using xylan and cellulose, and significantly more bacteria producing short-chain fatty acids (SCFAs), the association between the GM and non-transmissible chronic diseases have been widely investigated 19]. Among them, the link between the human GM and obesity, currently a major, global health concern, has received great attention [5]. It is well-known that modulation of the GM can have beneficial effects to controlling obesity, and several mechanisms that may contribute to microbiota-induced susceptibility to obesity and metabolic diseases have been proposed [20]. Changes in dietary patterns, specific functional foods, prebiotics or probiotics, have the potential to favorably influence host metabolism by targeting the GM and may be a useful approach for the

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management of obesity and metabolic conditions [20]. Various non-dietary factors, such as stress, age, exercise or climatic conditions, can also dramatically affect the human GM diversity and equilibrium [1,11,21]. Additionally, the ability of minor food components to modulate specific components of the GM has been acknowledged. These effects were cited for bacteriocins [22], dietary emulsifiers (DEs) [23], non-nutritive sweeteners (NNS) [24], essential oils (EOs) [25] and minor compounds from red meat [26]. However, the attention of the effect of these minor food constituents, such as food additives and trace contaminants on the GM has received less attention. Consequently, the present review aimed to provide an objective perspective of the current knowledge surrounding the effects of these minor foods constituents on the human GM, and, consequently, on human health.

2. Composition and evolution of human gut microbiota

There is a continuum increase in the number of bacterial cells present in the human gut that ranges from 10^1–10^3 bacteria per gram of contents in the stomach and duodenum, from 10^4–10^7 in the jejunum and ileum, culminating in 10^{11}–10^{13} in the colon, particularly in the distal part [12]. The GM also varies in composition depending on the location along the GI and axial depth (mucosal versus luminal) [27]. Globally, the microbial mass in the intestine represents about one kilogram of body weight and is essential to the metabolic demands required for the fitness of both, the microbe and the host [28].

Out of 53 known bacteria phyla on earth, only five to seven phyla (predominantly Firmicutes and Bacteroidetes, comprising 90% of the total) usually colonize the human gut [10]. Firmicutes (the most predominant phyla in people living in developed countries) comprise mostly Gram-positive bacteria with a DNA that has a low G+C content but also include Gram-negative bacteria. The Gram-negative bacteria are mainly represented by the *Bacteroides* genus in the human gut [29]. The relative proportions of these two dominant phyla vary and can be influenced by a range of factors, but most people have similar proportions of each [1]. Lesser (but also important) contributions from members of the Cyanobacteria, Proteobacteria, Actinobacteria, Fusobacteria and Verrumicrobia phyla comprise the rest of the community [28].

Bacteroidetes, *Faecalibacterium*, *Bifidobacterium* and *Eubacterium* are numerically the most important genera among the GM and may account for more than 60% of the bacteria present in human stool, but their relative abundance is highly variable across individuals [1,30]. *Clostridium*, *Enterobacteriaceae* and *Streptococcus* are also important but less numerous [1].

One metagenomic analysis suggested that the GM of each human is typified by one of three enterotypes, with each enterotype characterized by distinct dominant groups of microbes [31], namely *Bacteroides*, *Prevotella* and *Ruminococcus*. However, subsequent studies, including those of The Human Microbiome Project, have been unable to provide conclusive evidence that supports this concept [32,33].

The development of the human GM is a large and complex process that begins during the fetal age. Recent studies have reported that microbial contact is initiated throughout the course of fetal development and continues thereafter in an accelerated manner [32,34,35]. The diversity of the GM in the infant gut is initially very low, and the GM are generally aerotolerant, as the gut initially contains oxygen, however, after birth, they are replaced by anaerobes that are typical of the adult GM [3]. The GM alters considerably from birth to 6 months, when the GM appears to be relatively similar to the childhood-type population [35]. At this age, one of the most important factors contributing to the formation of the GM is the type of lactation [32,36]. The bacterial composition begins to converge toward an adult-like GM by the end of the first year of life and fully resembles the adult GM by 2.5–3 years of age [32,37,38]. In terms of ecological succession, the *Bifidobacterium*-dominated GM of the infant changes over time into the Bacteroidetes- and Firmicutes-dominated GM of the adult, which can be affected by several factors [27,31,32,39]. Among dietary factors, it was observed that subjects ingesting a diet particularly rich in protein and animal fat (such as the typical Western diet) were associated with the *Bacteroides* enterotype, whereas the GM of subjects ingesting more carbohydrates were dominated by the *Prevotella* enterotype [38]. An increase in the phylum Firmicutes and a decrease in the Bacteroidetes (mainly expressed as the

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Firmicutes/Bacteroidetes ratio; FBR) associated with obesity was observed in some, but not all studies [31]. Additionally, an increase of Actinobacteria in obese individuals was also reported [31]. These changes are probably not a mere consequence of obesity because GM obese phenotype can be transplanted into mice, indicating that the GM may have an active role in obesity pathogenesis [1,11].

Once the GM has reached maturity it remains mostly stable until old age, although some differences can be found in the GM of the elderly from that of young adults [3]. Particularly, Bacteroidetes phyla and *Clostridium* genus predominate in the GI of elderly people compared to higher proportions of Firmicutes in young adults [21]. Elderly people are also noted to have significant decreases in *Bifidobacteria*, *Bacteroides*, and *Clostridium* cluster IV [21]. Variability in community composition is greater in this age group than for adults and varies greatly among individuals, ranging from 3–92% for Bacteroidetes and 7–94% for Firmicutes [3,40]. This could be related to the greater number of morbidities associated with the elderly and the complex repertoire of drugs used to treat them that are likely to affect the microbiota [40].

3. Impact of the human gut microbiota with effect on human health

The GM is essential for several physiological functions associated with great impact on human health, affecting almost all organ systems that contribute to metabolic control. Thus, the GM modulates appetite and food intake [38], absorption of nutrients from the gut, hepatic steatosis, inflammation and triglyceride accumulation in adipose tissue [41], and fatty acid oxidation in skeletal muscle and the liver [38]. However, there is still limited knowledge on the exact mechanisms by which the GM affects human metabolism.

The GM express the enzymatic machinery to process otherwise non-digestible carbohydrates, such as fructooligosaccharides, galactooligosaccharides and inulin, and thus, release monosaccharides that can be used by the host for metabolic purposes [21]. In addition to the conversion of complex carbohydrates into absorbable substrates, the GM also benefits the human host by producing SCFAs, with great impact in the colonic epithelial cells maintenance, and vitamins, like vitamin K, as well as most of the water-soluble B vitamins, such as biotin, cobalamin, folates, nicotinic acid, pantothenic acid, pyridoxine, riboflavin and thiamine [12]. In contrast to dietary vitamins, which are absorbed in the proximal tract of the small intestine (SI), the predominant uptake of microbially produced vitamins occurs in the colon.

The GM also influences the host health status through the enzymatic transformation of bile acids, natural detergents with novel signaling functions including regulation of cholesterol synthesis and absorption, modulation of inflammatory responses, and energy homeostasis [21]. Moreover, the GM synthesizes amino acids, influences iron absorption, and is involved in the conversion of dietary polyphenolic compounds and in the bile acid biotransformation process [18]. The intestinal microbiota is able to transform potentially carcinogenic compounds, such as N-nitroso compounds and heterocyclic amines, and to activate bioactive compounds including phytoestrogens [18].

Globally, although the ideal healthy GM is not yet fully established, it is well known that the richness and diversity of bacterial species in the human gut may be an indicator of health, and consequently, alterations in GM can affect multiple health issues [27]. In this context, compositional and functional alterations in the GM have been linked to malnutrition [42], obesity and obesity-related diseases [6,11], cardiovascular disease [43], type 2 diabetes [44], inflammatory bowel disease [45], colorectal cancer [46], neurodevelopmental disorders [47] and aging-related diseases [48]. Considering the increasing global incidence of many of these conditions, changes in the lifestyle and diet in the post-industrialization/westernization era have been argued to contribute to their emergence by shifting the GM ecology [38].

Knowledge of the effects of specific microbial phyla is still limited. However, Firmicutes, from diverse families, namely *Clostridiales*, *Erysipelotrichaceae*, *Ruminococcaceae*, *Eubacteriaceae*, and *Lachnospiraceae* have been shown to be associated with healthy populations [21]. Additionally, certain bacterial genus such as *Bacteroides*, *Bifidobacterium*, *Clostridium* clusters XIVa/IV, *Eubacterium*, *Faecalibacterium*, *Roseburia* or *Lactobacillus* and even specific species, such as *Akkermansia muciniphila*,

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Faecalibacterium prausnitzii or *Roseburia intestinalis*, have been shown to prevent health disorders such as obesity or diabetes, or to improve immunity and inflammatory status [5,21,27,32].

4. Effect of minor food compounds on the human gut microbiota

Although dietary patterns have an important effect on the human GM, the individual effects of minor food compounds have been less investigated than diets with different proportions of macronutrients, such as fat, protein or carbohydrates. Micronutrients are pivotal for several health-related functions, like energy metabolism, cellular growth and differentiation, and organ and immune function [49]. A diet low in micronutrients, but not necessarily low in energy, is frequent in populations of low-income countries, buy may also be present in poverty-affected settings in middle- and high-income countries [49]. It is estimated that more than three billion people worldwide suffer from various types of micronutrient deficiencies (predominantly vitamin A, iron and zinc), with the majority being women and children [49]. Vitamin A can modulate the immune response of the intestine by direct interactions with immune cells of indirect modulation of the microbiota [16]. Iron deficiency or anemia is related to a depletion of *Lactobacillus* in women [7]. Moreover, even mild zinc deficiencies can profoundly impact growth and development, as well as impede immune differentiation and maturation [50]. Supplementation with high levels of zinc has been shown to result in an increase of *Lactobacillus* in the GM of weaned pigs [51]. Using chicks as a model, one study recently showed that zinc deficiency results in a remarkable change in the microbiota, with metabolic changes, such as decreased SCFAs output [50].

Various other dietary constituents, including various compounds belonging to polyphenols, also nourish colonic microbes [1]. Polyphenols are secondary metabolites found abundantly in a wide variety of foods, such as fruits, vegetables, herbs, seeds and cereals, and in beverages, such as coffee, tea, cocoa and wine [52]. The beneficial activities of polyphenols on the prevention of cancer and cardiovascular disease and, specifically, on the GM have been widely investigated in recent years [1,52]. Most polyphenols pass through the SI without being absorbed, thus encountering the GM, which colonizes the colon [52]. Once reached the colon the interaction polyphenols-GM results in a two-way mutual reaction. First, polyphenols are biotransformed *in vivo* by the GM that increases their bioavailability. Second, polyphenols modulate the composition of the GM mostly through the inhibition of pathogenic bacteria and the stimulation of beneficial bacteria [52]. Several phenolic compounds have been recognized as potential antimicrobial agents with bacteriostatic or bactericidal effects, and have various effects on bacterial species or genus [52]. About 90% of the dietary polyphenols escape digestion and absorption in the SI [53,54] and can have a significant influence on the microbial populations and their activities [55-57] but our understanding of the microbial bioconversion processes is limited [57].

Flavonols [57], quercetin [58,59], catechin and puerarin [59], anthocyanins [47], ellagitannins [60], resveratrol [61] and *trans*-resveratrol [58] are all reported to impact the activities of the GM. Quercetin supplementation resulted in an altered composition of the GM at different taxonomic levels, including the FBR and inhibiting the growth of bacterial species associated with diet-induced obesity, such as *Erysipelotrichaceae*, *Bacillus* spp., and *Eubacterium cylindroides* [58]. In other recent work, it was demonstrated that different types of flavonoids can modulate the growth of different phyla and genus from GM [59].

Hidalgo et al. [62] investigated the bacterial metabolism of malvidin-3 glucoside, gallic acid and a mixture of anthocyanins using an *in vitro* model of the human gut. The anthocyanins universally enhanced the growth of *Bifidobacterium* spp. and *Lactobacillus-Enterococcus* spp. significantly. Li et al. [60] demonstrated that ellagitannins can stimulate the growth of several bacterial genera with beneficial properties for human health, such as *Akkermansia muciniphila, Butyrivibrio, Escherichia, Lactobacillus* or *Prevotella*. Proanthocyanidins from grape seed can increase *Lachnospiraceae, Clostridiales*, *Lactobacillus* and *Ruminococcacceae* in female pigs [63].

In another work, Qiao et al. [61] found that resveratrol ameliorated the dysbiosis in the GM of mice induced by a high-fat diet. Specific effects included an increase in the FBR, significant inhibition of the growth of *Enterococcus faecalis*, and increased growth of *Lactobacillus* and

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Bifidobacterium. Flavonols can also increase the relative abundance of *Bifidobacterium* and *Lactobacillus* at the expense of potentially pathogenic bacteria, notably the *C. histolyticum* group [56]. Therefore, isoflavones markedly altered dominant bacterial communities, including the *Clostridium coccoides-Eubacterium rectale* cluster, *Lactobacillus-Enterococcus* group, *Faecalibacterium prausnitzii* subgroup, and *Bifidobacterium* genus [64].

Besides polyphenols, other minor compounds have been reported to modulate the GM and consequently, impact human health. Chaplin et al. [65] did not find any specific impact of high-fat diet on the abundance of *A. muciniphila*, they found that feeding mice with a high-fat diet enriched with conjugated linoleic acid increased the intestinal *A. muciniphila* levels, that was associated with several beneficial associations with metabolism [2].

Regarding to the micronutrients profile in human omnivores and vegans the studies did not show clear taxonomic shifts in the gut community of both collectives, however these studies revealed, in vegans, distinct profiles of bacterial metabolites in plasma in comparison with those of omnivores, as well as a reduced capacity to metabolize L-carnitine, present in red meats, to trimethylamine [26]. Colonic bacteria can hydrolyze choline to form dimethylamine and trimethylamine, which are precursors of dimethylnitrosamine [12], a potent hepatotoxin, and carcinogen.

Mice fed a high-fat diet with 0.25% sphingomyelin showed a higher relative phylogenetic abundance of the predominately Gram-positive Firmicutes phylum and significantly lower numbers of the Gram-negative Bacteroidetes phylum and some intestinal pathogens [66]. Comparing the minor bacterial phyla, these mice had a significantly higher relative abundance of the Gram-positive Actinobacteria phylum and less of the Gram-negative Tenericutes phylum [66]. Additionally, these mice had a significantly lower relative abundance of Gram-negative bacteria and a reciprocal increase in the predominately Gram-positive bacteria [66]. Milk sphingomyelin had a significantly relative abundance of the beneficial bacteria *Bifidobacterium* [66]. Interestingly, milk sphingomyelin tended to have a higher relative abundance of *Bacteroides*, one of the few microbes that synthesize and utilize sphingolipids [66].

A summary of previously published works regarding effects of food minor compounds in human GM can be seen in Table 1.

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Table 1. Recent works regarding the effects of micronutrients on the human gut microbiota (GM)

Reference	Study design	Micronutrients	Supplementation dosage	Main conclusion
Balamurugan et al. (2010) [67]	Observational study (8 anemic and 26 normohemic females)	Iron	-	Fecal *Lactobacillus* were significantly lower in anemic women
Chaplin et al. (2015) [65]	Animal experimentation (pigs)	Conjugated linoleic acids (CLA)	6 mg of CLA/day was given to mice consuming both a normal-fat diet and a high-fat diet	CLA supplementation exerted a prebiotic action on *Bacteroidetes/Prevotella* and *Akkermansia muciniphila*. However, it was not able to override the negative effects of a high-fat diet on *Bifidobacterium* spp.
Choy et al. (2014) [63]	Animal experimentation (pigs)	Proanthocyanidins	Diet containing 1% (w/w) of grape seed extract daily for 6 days	Dramatic increase in faecal *Lachnospiraceae*, *Clostridiales*, *Lactobacillus* and *Ruminococcacceae*
Etxeberría et al. (2015) [58]	Animal experimentation (rats)	Polyphenols	*Trans*-resveratrol (15 mg/kg body weight/day], quercetin (30 mg/kg/day) or a combination of both polyphenols at those doses	Quercetin attenuated the *Firmicutes/Bacteroidetes* ratio and inhibited the growth of *Erysipelotrichaceae*, *Bacillus* and *Eubacterium cylindroides*. *Trans*-resveratrol supplementation alone or in combination with quercetin scarcely modified the GM
Hidalgo et al. (2012) [62]	*In vitro* model of human gut	Malvidin-3-glucose, gallic acid and a mixture of anthocyanins	Gallic acid (150 mg/L and 1000 mg/L), malvidin-3-glucoside (20 mg/L and 200 mg/L), and enocianin (4850 mg/L and 48500 mg/L)	All the anthocyanins tested significantly enhanced the growth of *Bifidobacterium* spp. and *Lactobacillus–Enterococcus* spp.
Huang et al. (2012) [59]	*In vitro* model of the human gut	Flavonoids (quercetin, catechin, puerarin)	Each flavonoid at 0.15g/L	Catechin and puerarin presented different activities on regulating the GM, but all increased GM diversity
Li et al. (2015) [60]	Uncontrolled study including 22 healthy human volunteers	Ellagitannins	Pomegranate extract at 1000 mg/day for 4 weeks	Ellagitannins from pomegranate stimulated *Akkermansia muciniphila, Butyrivibrio, Enterobacter, Escherichia, Lactobacillus* and *Prevotella* and inhibited *Collinsella* in fecal samples
Martin et al. (2012) [56]	Case-controlled study including 22 healthy human volunteers	Flavonols	Dark chocolate at 50 g/day for 1 week	Cocoa flavonols increased the relative abundance of *Bifidobacterium* and *Lactobacillus* at the expense of potentially pathogenic bacteria, notably the *C. histolyticum* group
Norris et al. (2016) [66]	Animal experimentation (mice)	Sphingomyelin	High-fat diet with 0.25% of milk sphingomyelin added 45% Kcal as fat	Decrease in Gram-negative bacteria, such as Bacteroidetes or Tenericutes phyla and increase in Gram-positive bacteria, such as Firmicutes and *Actinobacteria* phyla
Qiao et al. (2014) [61]	Animal experimentation	Resveratrol	200 mg/kg per day	Resveratrol increased GM dysbiosis induced by a high-fat diet, with an increase in the FBR, *Lactobacillus*

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	(mice)			and *Bifidobacterium* growth and a significant decrease in *Enterococcus faecalis*
Reed et al. (2015) [50]	Animal experimentation (chicken)	Zinc	Zinc oxide supplementation at 42 μg/g or 2.5 μg/g	The zinc-deficient group had a significantly lower cecal microbial diversity
Starke et al. (2014) [51]	Animal experimentation (pigs)	Zinc	57 (low) or 2425 (high) mg/kg zinc oxide for 5 weeks	Pronounced reductions were observed for *Enterobacteriaceae* and the *Escherichia* group as well as for *Lactobacillus* spp. and for three of five studied *Lactobacillus* spp.
Tzounis et al. (2011) [55]	Case-control study including 22 healthy human volunteers	Cocoa flavonols	The high-cocoa flavonol (HCF) group received 494 mg cocoa flavonols/day), while the low-cocoa flavanol and low-cocoa flavanol group received 23 mg cocoa flavonols/day, for 4 weeks	Consuming the HCF drink for 4 weeks significantly increased the *Bifidobacterium* and *Lactobacillus* populations but significantly decreased the *Clostridia* counts in fecal samples

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5. Effects of food additives on human gut microbiota

An important change in human diets since the mild-20th century is the increasing consumption of food additives that are incorporated into almost all processed foods, often to aid stability, shelf-life, taste, and texture improvement, particularly in processed foods. The primary basis for approving the use of these agents is the notion that they do not cause acute toxicity at concentrations reasonably greater than their approved concentrations. However, only few prospective interventional human studies address possible causal effects of additives on the human GM, presumably due to difficulties in allocation of cohorts of healthy individuals who have not been previously exposed to food additives, and the need for robust stratification of potentially confounding factors, such as genetics, lifestyle and dietary patterns [68]. Consequently, researchers have turned to animal models to study the effect of food additives on the GM. Recent studies have demonstrated that the consumption of NNS and DEs can alter the GM, resulting in intestinal inflammation and favoring the development of the metabolic syndrome [68,69] (Table 2).

The DEs seem particularly disconcerting. Most processed foods contain one or more DEs that allow such foods to maintain desired textures and avoid separation into distinct parts. Two DEs, namely carboxymethylcellulose and polysorbate 80, had been demonstrated to promote bacterial overgrowth in the murine SI and facilitate translocation of bacteria across a model gut epithelia [70]. Some authors have suggested that DEs may be one specific factor resulting from industrialization that has resulted in a reduction of GM diversity, altered host-microbiota interactions and, consequently, have contributed to the increased incidence of metabolic syndrome and other inflammatory diseases in industrialized societies [70,71]. The ingestion of DEs, such as carboxymethylcellulose or polysorbate 80, dramatically reduced the mucus layer thickness and was involved in the onset of intestinal inflammation, obesity, and diabetes. These effects were also associated with an increased food intake, from an unknown origin [70].

As a result of the many negative health conditions associated with the intake of excessive sugar, there has been an upsurge in the consumption of NNS as an alternative [24]. NNS are synthetic compounds that are several hundred-fold sweeter than sucrose. Thus, they can be used in small amounts with negligible added caloric value. NNS are excreted unchanged from the mammalian body, and are, therefore, considered metabolically "inert" [24]. Theoretically, NNS would only aid in weight loss if compensatory sugar intake did not occur. However, rats administered liquids containing saccharin, consumed more food and gained more weight compared to rats given liquids containing glucose [72]. The common perception that NNS may promote weight loss by reducing calories is misguided because consumption of saccharin-sweetened liquids increased overall food intake [24]. Furthermore, positive correlations between NNS consumption and increased body mass index in children and adolescents have been reported in several observational studies [24,73].

The effects of NNS on the GM could be due to the bacteriostatic effects of the NNS, saccharin, sucralose, aspartame and stevia [68,74,75]. Data from studies in animals [74,75] and from a small study in human subjects [68] suggests that the bacteriostatic effects of NNS are not limited to the microbial inhabitants of the mouth, but extend to those in the gut, thereby affecting the host metabolic phenotype and disease risk [76]. Pioneer work showed that 12 weeks of exposure to Splenda significantly altered the GM composition by decreasing beneficial bacteria and was associated with weight gain in rats [74]. In a recent work, it was confirmed and extended these findings by identifying a microbe-mediated mechanism by which NNS might influence metabolism [68], inducing higher glucose intolerance, mediated by alterations in the GM.

Consistent with previous findings showed that 8 weeks of aspartame exposure in a dose equivalent to human subjects consuming 2–3 diet soft drinks per day, perturbed the GM and resulted in elevated fasting glucose levels and impaired insulin tolerance in rats [68,75].

The effects of other additives on the human GM have also investigated. For instance, other additives reported to significantly alter the GM are EOs, which were used to prevent the growth of pathogenic bacterial species that are generally more sensitive to EOs than most commensal bacteria [25]. It was demonstrated that several EOs (mainly thymol), selected for their effectiveness against gut pathogens (*C. difficile*) did not have significant effects on the abundance of *F. prausnitzii*, which

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plays an important anti-inflammatory role in the gut [25]. In particular, EOs may have potential use as an adjunct to chemotherapeutic agents used to treat colorectal cancers. Patients receiving chemotherapy for cancer treatments suffer from gastrointestinal disturbances due to damage to the mucosal cells of the GI. The use of antibiotics against infections disrupts the ecological balance and increases the risk of bacterial infections, such as the overgrowth of *C. difficile* [25]. Their study revealed that *C. difficile* proliferated at the expense of decreased *Bifidobacterium*, *Lactobacillus*, *Veillonella* and *F. prausnitzii* in cancer patients after chemotherapy with or without antibiotic treatments. Consequently, EOs might be exploited as prophylactic agents and as adjuncts in chemotherapy to decrease the use of antibiotics that have adverse effects on commensal bacteria, including *Bifidobacterium* spp. and *F. prausnitzii* [77].

Alginate oligosaccharides were reported to have antifungal, anti-inflammatory and immunomodulatory activities [78]. Like other edible dietary fibers, alginate and its oligomer derivatives are resistant to digestion by human endogenous enzymes, but can be used to a large extent of enzymes produced by the human GM [78]. It was demonstrated that the alginate oligosaccharides enhanced the growth of intestinal *Bifidobacterium* and *Lactobacillus* of rats after feeding for 2 weeks [78].

Other additives that were reported to can significantly alter GM were emulsifiers. In a study carried out in mice, administration of Polysorbate-80 (P80) and carboxymethylcellulose (CMC) in concentrations commonly used in foods they not only alter the composition of the GM but also the location [23]. This modification induces intestinal inflammation, which usually promotes the development of inflammatory bowel disease such as ulcerative colitis. A previous study, also carried out in mice, showed as the same emulsifier, P80 enhances the traslocation of *E. coli* across M-cells [79]. An increase in numbers of *E coli* have been found in association with Crohn's mucosa. There are studies showing that the *E. coli* traslocation can increase in 59 folds. Thus, this emulsifier may contribute to the impact of dietary factors on Chron's disease pathogenesis.

The possibles mechanisms to explain effects of emulsifiers in Chron´s disease pathogenesis is explained [79]. These ingredients are broken down on passage through the small intestine and their detergent effects in the distal colon and ileum may arguably be small compared with the natural effects of bile acids. In some cases, such as for the emulsifier lecithin, the intestinal barrier function can be enhanced. However, in other cases, as for polysorbate-60 and 80 this function is altered by increasing permeability and causing cell traslocation.

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Table 2. Recent works regarding the effects of food additives on the human gut microbiota (GM)

Reference	Study design	Additive	Supplementation dosage	Main conclusion
Abou-Donia et al. (2008) [74]	Animal experimentation (rats)	Splenda	100, 300, 500, or 1000 mg/kg for 12 weeks	Total anaerobes, bifidobacteria, lactobacilli, Bacteroides, clostridia, and total aerobic bacteria were significantly decreased. No significant changes were found in the *Enterobacteriaceae*
Chassaing et al. (2015) [23]	Animal experimentation (mice)	Carboxymethylcellulose and polysorbate-80	1% of each emulsifier for 12 weeks	Reduction in the microbial diversity, Bacteroidales, Verrucomicrobia phyla (particularly *Akkermansia muciniphila*) and enriched mucosa-associated inflammation-promoting Proteobacteria
Cowan et al. (2013) [80]	Animal experimentation (rats)	Aspartame	Chow and high-fat feed added with 0.4 g/100 mL of aspartame in water for 8 weeks	Increase in total bacteria associated with aspartame addition, and reductions in *Lactobacillus* and *Bacteroides*
Daly et al. (2014) [81]	Animal experimentation (pigs)	Saccharin	Diet supplemented with 0.015% saccharin + neoesperidin dihydrochalcone	Saccharin + neoesperidin dihydrochalcone dramatically increased the cecal population abundance of *Lactobacillus*
Palmnäs et al. (2014) [75]	Animal experimentation (rats)	Aspartame	5–7 mg/kg/day for 8 weeks	Aspartame increased total bacteria, Enterobacteriaceae and *Clostridium leptum* in the feces, and attenuated the increase in Firmicutes/Bacteroidetes ratio
Suez et al. (2014) [68]	Animal experimentation (mice)	Saccharin	0.1 mg/ml in water for 11 weeks	Saccharin induced an increase of the Bacteroidetes and reduction in Firmicutes
Rettig et al. (2014) [82]	*In vitro* trial	Sucralose	1.1–11 mg/kg	Sucralose had little effect on *E. faecalis* and *C. sordellii*, while there was a concentration- dependent inhibition of the growth of *Bacteroides*, *B. fragilis* and *B. uniformis*
Thapa et al. (2015) [25]	*In vitro* model of the human gut	Thymol, nerolidol, eugenol, methyl isoeugenol and geraniol	100–500 mg/kg	Thymol and geraniol suppressed pathogens, such as *C. difficile*, with no concern for beneficial commensal colonic bacteria in the distal gut

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6. Toxic compounds produced by the metabolism of gut microbiota

6.1. Food ingredients

In addition to their action on certain populations of the GM, some ingredients can be metabolized by gut microorganisms and exert potentially toxic effects to their consumers. In particular, alcohol can be metabolized by bacteria to aggravate their intrinsic negative effects. Thus, oral bacteria, such as streptococci, have the capacity to convert ethanol in wine to acetaldehyde, which is an *in vitro* and *in vivo* genotoxin and a recognized human carcinogen [64,83]. Furthermore, the GM is suggested to play an important role in alcohol-induced liver injury, apparently through dysbiosis of the intestinal ecosystem caused by alcohol intake [83].

Fermentation of protein by large bowel bacteria results in the production of energy for colorectal tissues and bacteria and promotes cellular mechanisms that maintain tissue integrity [1]. However, some protein fermentation products, such as ammonia, phenols and hydrogen sulfide, can also be toxic [1]. These fermentation products can cause a significant decrease in cancer-protective metabolites (e.g. butyrate) and the greatest formation of hazardous metabolite profiles that is probably detrimental to colonic health [84]. Additionally, fermentation of protein sources by the GM can also increase putrefactive fermentation products [1], life sulfide, which is positively associated with greater DNA damage in the colonic mucosa, particularly when dietary levels of fermentable carbohydrates are low [85]. Although ammonia is a well-known toxin, it is used as a nitrogen source by the microbiota and most is excreted via stool or absorbed in the gut and eliminated in the urine. Other bacterial metabolic products such as trimethylamine –N-oxide, produced from L-carnitine, abundant in red meat, could increase the risk of atherosclerosis [26].

Devroka et al. [17] indicated that consumption of a diet high in saturated (milk-derived) fat can markedly alter the conditions for gut microbial assemblage and promote the expansion of a sulfite-reducing pathobiont *Bilophila wadsworthia*, resulting in the increased incidence of colitis in genetically susceptible rodent models. Furthermore, it was reported that the occurrence of renal injury in infants and children exposed to melamine-tainted milk in China could also be attributed to the metabolism of the GM [86]. Certain gut bacterial species, like *Klebsiella terrigena*, can convert melamine to cyanuric acid, which then forms complex precipitates that lead to kidney stone formation and causes renal toxicity [86]. A summary of previously published work, describing toxic compounds produced by its metabolization by GM, in both food ingredients and contaminants, can be seen in Table 3.

6.2. Food contaminants

Another group of compounds that can be metabolized by the GM and cause harmful effects are contaminants, such as drugs, heavy metals or environmental chemicals [96]. An interesting study showed how the GM has the ability to inactivate drugs delivered into the intestine, with the potential to generate toxic compounds, like hydrogen sulfide [18]. The gut normally converts luminal hydrogen sulfide to thiosulfate, which can be further oxidized to tetrathionate. High concentrations of hydrogen sulfide severely inhibit cytochrome 1c oxidase, blocking mitochondrial activity [18,94]. Regarding biotransformation of heavy metals by the GM, Pinyayev et al. [89] reported that anaerobic microbiotas of the mouse cecum convert arsenate into oxyarsenicals and thioarsenicals. Additionally, it was reported that exposure to mercury altered the bacterial community in the gut of a terrestrial isopod [87].

Environmental contaminants may be poorly absorbed after ingestion, and subsequently can reach the distal SI and caecum by peristalsis. Additionally, environmental chemicals (or their metabolites) may also be excreted in the bile [96]. There is increasing evidence that chronic exposure to environmental chemicals through the diet, particularly persistent organic pollutants, may promote the development of obesity and type 2 diabetes in humans, even without inducing dysbiosis [96]. Of particular interest is the role of the aryl hydrocarbon receptor, which is bound and

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activated by a variety of persistent organic pollutants including coplanar polychlorinated biphenyls and halogenated aromatic hydrocarbons [96]. For instance, it was recently reported that a persistent organic pollutant, 2,3,7,8-tetrachlorodibenzofuran, can dramatically alter the GM by shifting the FBR, increasing *Butyrivibrio* spp. and decreasing *Oscillibacter* spp. These changes in the GM were associated with altered BA metabolism and subsequent host metabolic disorders as a result of an altered hepatic lipogenesis, gluconeogenesis, and glycogenolysis [96].

Conversely, the GM can regulate the expression of cytochrome P450 enzymes, which are involved in the metabolism of a variety of environmental chemicals [97]. Polycyclic aromatic hydrocarbons are among the most widespread organic pollutants and can be transformed by the GM to estrogenic metabolites [90]. Furthermore, it has been shown that the rat and human GM could regenerate benzo(a)pyrene from its hepatic conjugate, reversing the endogenous detoxification process, which is of potential toxicological relevance [97]. Choi et al. [87] reported that after exposure to polychlorinated biphenyls in mice, the most striking change in the intestinal microbial profiles was a decrease in bacterial species.

Other environmental chemicals, for example, pesticides or herbicides, can also exert increased harmful effects on human health via the action of the GM. Indeed, chronic exposure to chlorpyrifos, an organophosphate insecticide commonly used to treat fruit and vegetable crops and vineyards has been shown to induce dysbiosis of the GM in both human and rats and was associated with the proliferation of *Bacteroides* sp. and decreased levels of *Lactobacillus* sp. and *Bifidobacterium* sp. [88]. Glyphosate, the most widely used herbicide worldwide, has been shown to have important effects in poultry GM [92]. The sensitivity to glyphosate is dependent on the bacterial strain. Some typical pathogens, such as *Salmonella* or *Clostridium*, are highly resistant, whereas beneficial bacteria, like *Lactobacillus* spp. or *Bifidobacterium* spp. are moderately or high susceptible. No trials were performed using human models, but if it were demonstrated that glyphosate acts similarly in human GM, this would be of a toxicological relevance [96].

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Table 3. Recent works regarding foods that can become toxic by the metabolism of the human gut microbiota (GM)

Reference	Study design	Food/substance	Dosage	Main conclusion
Canesso et al. (2014) [83]	Animal experimentation (mice)	Alcohol	10% v/v in drinking water for 7 days, plus an additional oral gavage of 5 mg/kg on day 7	The GM plays an important role in alcohol-induced liver injury, apparently through dysbiosis of the intestinal microbial ecosystem caused by alcohol intake
Choi et al. (2013) [87]	Animal experimentation (mice)	Mixture of polychlorinated biphenyls (PCBs) congeners	150 μmol/kg for 2 days	PCBs decreased the levels of Proteobacteria and induced substantial changes in the gut microbiome, which may then influence their systemic toxicity
Devroka et al. (2012) [17]	Animal experimentation (mice)	Diets containing different types of fat	Low-fat, saturated milk fat, and saturated lard fat for 5 weeks	Milk-derived-fat-promoted increased the availability of organic sulfur used by sulfite reducing microorganisms like *Biophila wadsworthia*
Humphreys et al. (2014) [85]	Randomized cross-over design, 23 human volunteers	High red meat diet	300 g/day lean red meat, or the same plus 40 g/day butylated high-amylose maize starch for 4 weeks	Fecal propionate and butyrate increased with the diet. Resistant starch consumption reduced the risk associated with a high red meat diet
Joly et al. (2013) [88]	Animal experimentation (rats)	Chlorpyrifos	1 mg for 30 days	Chronic, low-dose exposure to chlorpyrifos was found to induce dysbiosis in the microbial community with the proliferation of *Bacteroides* sp. and decreased levels of *Lactobacillus* and *Bifidobacterium* spp.
Koeth et al. (2013) [26]	Animal experimentation (mice)	Normal chow diet and L-carnitine diet		Mice placed on an oral antibiotic cocktail to suppress intestinal microbiota showed marked reductions in plasma trimethylamine and trimethylamine oxide levels
Pinyayev et al. (2011) [89]	Animal experimentation (mice)	Arsenic	Cecal content of mice was added with 0, 200, 1000 and 2000 μg/kg arsenic	Thioarsenicals were found in soluble and particulate fractions of the reaction mixtures, suggesting interactions with anaerobic microbiota
Van de Wiele et al. (2005) [90]	*In vitro* model of human gut	Polycyclic aromatic hydrocarbons (PAHs)	Hypothetical soil ingestion of 5 g/day	PAHs biotransformation potency of colon microbiota suggests that the current risk assessment may underestimate the risk from ingested PAHs
Russell et al. (2015) [91]	Crossover trial with 17 obese males	Different dietary patterns	4 weeks with each weight-maintenance diet, high-protein and moderate carbohydrate diet and high-protein and	Weight-loss diets high in protein but reduced in total carbohydrates and fiber resulted in a significant decrease in fecal cancer-protective metabolites and increased concentrations of hazardous metabolites, such as phenylacetic acid and N-nitroso compounds

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			low-carbohydrate diet	
Shehata et al. (2013) [92]	*In vitro* trial	Glyphosate	0.05, 0.15, 0.075, 0.3, 0.6, 1.2 and 2.4 mg/ml for 5 days	Reduction of beneficial bacteria, such as some *Bifidobacterium* spp. or *Lactobacillus* spp. that could disturb the normal gut bacterial community, whereas limited effect was shown on the intestinal pathogens
Zhang et al. (2015) [93]	Animal experimentation (rats)	2,3,7,8-tetrachlorodibenzofuran	24 μg/kg for 5 days	Dietary 2,3,7,8-tetrachlorodibenzofuran altered the GM by shifting the Firmicutes/Bacteroidetes ratio. The cecal content was enriched with *Butyrivibrio* spp. but depleted in *Oscillibacter* spp. These changes in the GM were associated with altered hepatic lipogenesis, gluconeogenesis, and glycogenolysis
Jia et al. (2013) [86]	Animal experimentation (rats)	Melamine	0.2 mg/kg	Melamine is converted to cyanuric acid *in vitro* by *Klebsiella terrigena* cultured from normal rat feces. Rats colonized by *K. terrigena* showed exacerbated melamine-induced nephrotoxicity
Vermeiren et al. (2012) [94]	Animal experimentation (mice)	Nitric oxide (NO)	Daily intrarectal bolus treatment with an NO donor in two doses + 4% dextran sodium sulfate	NO-producing microorganisms in the gut lumen should be considered a modulating process during colitis
Wang et al. (2011) [95]	Animal experimentation (mice)	Nitrogen-rich diet	Chow control diet or diet supplemented with 1.0% betaine, 1.0% choline, 0.12% trimethylamine *N*-oxide or 1.0% dimethylbutanol for 3 weeks	Mice fed diets supplemented with trimethylamine species (choline or trimethylamine oxide) showed increased peritoneal macrophage cholesterol content and raised plasma levels of trimethylamine oxide

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7. Specific effects of antibiotics on the human gut microbiota

The GM has also been documented to actively participate in drug metabolism and multiple biotransformations of clinical drugs performed by intestinal bacteria, including reduction, hydrolysis, dehydroxylation, acetylation, deacetylation and deconjugation, have been reported [98]. When these compounds are orally administered; they can be transformed to bioactive, bioinactive, or toxic metabolites by intestinal microbiota before their absorption into the blood [99].

Several drugs can modulate the GM. Although it was reported that other pharmacological treatment, such as antidiabetic medication, can alter the GM [100], the drugs that primarily play the most significant action on the GM are the antibiotics [71,91,101]. Antibiotic administration, especially in the case of infants [102-106], in whom their use has been related to higher predisposition to infant obesity. The effects of antibiotics on the GM have been investigated actively in recent years, mainly using experimentation animals exposed to various concentrations of antibiotics to evaluate how they affect the microbiota and thus its microbiome. A summary of previously published work, describing antibiotic effects on the GM of experimental animals and humans, can be seen in Table 4.

As a general rule, it was reported that antibiotic intake in mice increased adiposity [4,20,106,110,111], and thus favored the development of obesity and type II diabetes [71,102], besides affecting normal metabolic activity, hormonal and immune development. However, antibiotic treatment does not always display adverse effects on the GM of experimental animals. Indeed, in some instances, antibiotic treatment improved the insulin response in Bio-Breeding diabetes-prone rats [121].

Antibiotics are one of the most prescribed drugs in human medicine, particularly in pediatrics and neonatal nursing in developed countries [104,111]. The effect of these drugs on the human GM, both during and after the treatment has been widely investigated in recent years, although it is not yet fully understood [29]. Although it is accepted that the intake of antibiotics produces dysbiosis, according to various authors, depending on factors, like the type of consumed antibiotic, dose, duration of treatment and the individual's response, antibiotics may slightly reduce, drastically reduce, or even increase the amount and diversity of our microbiota [27,108].

Antibiotics exert very different actions on the individual groups that constitute the GM. Overall, for a variable period after antibiotic treatment ceases, the microbiota usually regains its original composition. However, some bacterial species have been reported to irreversibly disappear in certain individuals [3]. This can influence the health of the host, particularly if the bacterial group that is decimated, affects a physiological health-related function [3].

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Table 4. Effects of antibiotic intake on the human gut microbiota (GM)

Reference	Study design	Antimicrobial	Dosage	Main conclusion
Ajslev et al. (2011) [4]	Prospective trial in 28,354 mother–child days for 7 years	Different antimicrobials	Several antibiotics and doses depending on the type of disease and patient characteristics	Early exposure to antibiotics increased the risk of being overweight in later childhood by decreasing the diversity of the GM
Arboleya et al. (2015) [108]	Prospective trial in 27 preterm infants and 13 full-time babies	Different antimicrobials	Several antibiotics and doses depending on the type of disease and patient characteristics	Prematurity and perinatal antibiotic administration strongly affect the initial establishment of microbiota, and caused lower percentages of *Lactobacillaceae* or *Bacteroidaceae* and increased *Enterobacteriaceae*
Azad et al. (2014) [103]	Retrospective cohorts study in 1-year-old babies	Penicillin, cloxacillin, cephalexin, cefadroxil or erythromycin	Different antibiotic treatments received during the first year of life	Exposure to antibiotics was associated with an increased risk of being overweight with central adiposity in pre-adolescence
Bailey et al. (2014) [102]	Cohort study in 64,580 children	Different antimicrobials	Several antibiotics and doses depending on the type of disease and patient characteristics	Repeated exposure to broad-spectrum antibiotics at ages 0–23 months is associated with early childhood obesity
Cho et al. (2012) [109]	Prospective trial in animal models (mice)	Penicillin, vancomycin, tetracycline or vancomycin + penicillin	Subtherapeutic dosages at 1 μg/g body weight per day	Antibiotic treatment induced significant changes in GM, increased adiposity and modified lipid metabolism and cholesterol
Cox et al. (2014) [110]	Prospective trial in animal models (mice)	Penicillin	Subtherapeutic dosages	Modified the GM and induced long-term changes in the metabolism of the host, inducing obesity
Dethlefsen et al. (2011) [111]	Prospective trial in 3 people before and after antibiotic treatment	Ciprofloxacin	1 g/day for 5 days	Ciprofloxacin treatment reduced the GM diversity, with significant effects on 1/3 of the bacterial taxa
Greenwood et al. (2014) [111]	Observational study in 74 infants	Ampicillin and gentamicin	Various dosages and treatment and durations	Infants who received 5–7 days of antimicrobials in the first week had an increased relative abundance of *Enterobacter* and lower bacterial diversity in the second and third weeks of life

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Jakobson et al. (2010) [113]	Prospective trial on 6 patients	Clarithromycin + metronidazole	250 mg + clarithromycin and 400 mg metronidazole	Antibiotic treatment affected the GM by decreasing Actinobacteria and this disturbance on the GM persisted after 4 years
Mikkelsen et al. (2015) [114]	Prospective study in 12 males	Vancomycin, gentamicin and meropenem	500 mg vancomycin, 40 mg gentamicin and 500 mg meropenem	Antibiotic treatment caused significant shifts in the GM. Nevertheless, the changes observed did not have important effects on glucose metabolism
Murphy et al. (2014) [105]	Retrospective cohort study of 74,946 children with asthma and /or allergies	Different antimicrobials	Several antibiotics and doses depending on the type of disease and patient characteristics	Exposure to antibiotics during the first year of life was associated with an increase in the body mass index of 5–8-year-old children
Panda et al. (2014) [29]	Prospective study in patients with no digestive diseases	Different antimicrobials	Several antibiotics and doses depending on the type of disease and patient characteristics	Both fluoroquinolones and beta-lactam reduced the GM diversity in more than 25% of the patients
Perez-Cobas et al. (2013) [115]	*In vitro* model of the human gut	Ampicillin + sulbactam and cefazolin	Ampicillin + sulbactam on first days and intravenous cefazolin during 14 days	Antibiotic treatment caused a marked decrease in Bacteroidetes and increase in Firmicutes
Robinson et al. (2010) [116]	Prospective trial in animal models (mice)	Vancomycin	100 mg/L in drinking water	Different antibiotics had specific effects on the GM
Russell et al. (2015) [91]	Prospective trial in animal models (mice)	Vancomycin or streptomycin	200 mg/L in drinking water	Vancomycin caused a loss in Bacteroidetes, which were largely replaced by Firmicutes, Paenibacillaceae, Verrucomicrobia (specifically *Akkermansia*), and *Enterobacteriaceae*. In contrast, streptomycin increased the Bacteroidetes, particularly *Porphyromonadaceae* and *Bacteroidaceae*
Trasandre et al. (2013) [106]	Observational study in 11,532 children	Different antimicrobials	Several antibiotics and doses depending on the type of disease and patient characteristics	Exposure to antibiotics during the first 6 months of life was associated with consistent increases in body mass from 10–38 months of age
Thuny et al. (2010) [117]	Observational study in 96 males	Vancomycin plus other antibiotics	Different doses depending on the type of disease and patient characteristics	Vancomycin plus gentamicin treatment increased the risk of obesity in men. High levels of *Lactobacillus* were found, possibly related to the use of vancomycin as a growth promoter

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Zhang et al. (2013) [118]	Prospective trial in animal models (mice)	Tetracycline and ampicillin	50 mg/kg or 2 mg/kg (for tetracycline) and 30 mg/kg (for ampicillin)	Antibiotic oral administration had important effects on the selection and extent of antibiotic resistance genes
Vrieze et al. (2014) [119]	Randomized controlled trial in 20 obese males	Vancomycin	500 mg for 7 days	Vancomycin reduced fecal microbial diversity with a decrease in Gram-positive bacteria (mainly Firmicutes) and a compensatory increase in Gram-negative bacteria (mainly Proteobacteria)
Van Vleck Pereira et al. (2016) [120]	Prospective trial in animal models (calves)	Ampicillin, ceftiofur, penicillin and oxytetracycline	0.005, 0.01 and 0.3 mg/ml and 0.1 g/ml, respectively from birth to weaning	Antibiotic residues resulted in discriminate GM communities, although they did not result in disruption of the taxonomic levels above the genus

Cho et al. [109] found a significant increase in the FBR as a result of the administration of beta-lactams and vancomycin. An increase in this ratio, as explained previously in this review, is associated in diverse studies with obesity and other metabolic disorders. Other authors [110] found significant decreases in the taxa associated with beneficial health properties, such as *Lactobacillus* spp. and *Bifidobacterium* spp. and significant increases of *Enterobacteriaceae* family that includes many genres considered potentially pathogenic. Other authors [91], treated mice with antibiotics, such as amoxicillin, metronidazole, cefoperazone, and a combination of all three. As a result, the Proteobacteria and, in particular, the *Enterobacteriaceae*, become dominant in the intestinal tract of the treated mice, accounting for 73% of the total microbiota. Two weeks after ceasing the antibiotic treatment, the microbiota of these animals recovered a relatively low proportion of Proteobacteria (5.77%), although it remained considerably more abundant than the percentage of the total microbiota representing this phylum in untreated mice (1.2%).

Indeed, although Proteobacteria usually represent about 15% of the intestinal microbiota, they accumulate more than 35% of the antibiotic resistance genes contained in the microbiome. In contrast, despite representing 31% of the total microbiota, *Bacteroidetes* accumulate only 6% of the antibiotic resistance genes [122]. Hence, it is highly feasible that an antibiotic treatment can cause less decline in the population of Proteobacteria (or even increase, occupying the space left by other bacterial groups more sensitive to the action of the antimicrobial) than *Bacteroidetes*, for instance. Similarly, it is also reasonable that once the Proteobacteria reach a high proportion within the microbiota, before gradually declining, its population will be maintained at high levels compared to prior to the administration of the antimicrobial.

Another study developed in experimental animals showed as after treatment with cefoperazone (a broad-spectrum antibiotic), there was a significant loss of microbial diversity, without recovery, even at six weeks post therapy [104,123]. In another research work [91], in which mice were given vancomycin or streptomycin in their drinking water, no significant changes regarding the action of streptomycin were found, while vancomycin was associated with significant variations in both the bacterial load and diversity. An almost total elimination of Bacteroidales and a marked enrichment of *Lactobacillus* was observed.

However, humans have a greater variation in diet and lifestyle than experimental mice, which introduces factors affecting the recovery of metabolic disturbances or susceptibility to weight gain [110]. Hence, the influence of antibiotics on the GM of humans, particularly children, have been studied. Children are often the most exposed to antibiotic treatments within the human population and typically experience the greatest effects [104]. Indeed, some reports suggest that exposure to antibiotics within the first 6 months of life predisposes the individuals to a significant increase in body mass in later life [4,20,106]. However, other authors found conflicting results, suggesting important differences according to the antibiotic regimens, their routes of administration, the choice of methods of statistical analysis, or other uncontrolled factors [104].

Similarly, treatment of preterm and low birth weight infants with a variety of antibiotics, including penicillin, ampicillin, cephalexin, gentamicin, amikacin, erythromycin, vancomycin, clindamycin and lincomycin, have been linked to an increase in *Enterobacteriaceae*, in conjunction with a decrease in healthy microbiota, such as *Bifidobacterium*, *Bacillus*, and *Lactobacillus* [108,110,112].

Antibiotic treatments can also significantly alter the microbiota composition of the adult GI, causing a decrease in the microbial diversity to between one-quarter to a third of the pre-antibiotic state [104]. However, in this stage of life, the GM is relatively strong and, in most instances, recovers after several weeks of ceasing the antibiotic treatment [29]. However, other studies have shown that after cessation of treatment, the microbiota requires several months to fully recover [29,111,113,124]. However, in some cases, it has even demonstrated that some bacterial groups eliminated by an antibiotic treatment not reappear again in several years after discontinuation of treatment [3,119,125]. These effects can be agudized in elderly people, in whose GM is less diverse compared to younger adults and a more unstable balance that can easily lead to the emergence of various pathologies [40,126].

Interestingly, little attention has been paid to the intake of antibiotics present in foods at low concentrations. Only a few works have focused on the effects of low concentrations of antibiotics on the GM [109,110,120]. This is surprising because antibiotics are the most widely used drugs in the livestock industry in the world [127] and their residues can reach humans through animal feeds. Paradoxically, while humans are interested in modulating their microbiota to aid in weight loss, producers of animal feed have used antibiotics for decades to increase the weight gain of the animals. Antibiotics in livestock production are incorporated in animal feed either as growth promoters in countries where such use is allowed [110,128] or as prophylactic therapeutic agents in the European Union and other countries where antibiotic use as growth promoters is banned. Importantly, these antibiotic effects are not limited to oral administration, but may also be present and, therefore, have effects on microbiota when administered parenterally [125].

It has also been shown that upon contact with antibiotics, the GM is perhaps the most accessible reservoir of genes encoding antibiotic resistance due to their high density within the gut ecosystem, which can have important consequences for human health [125]. The GI is also an open system, which incorporates everyday bacteria from the environment [129]. These incoming bacteria often possess antibiotic resistance genes, and besides being a potential risk to the host, because these resistance encoding genes can be transferred to the host.

8. Conclusions

The vast majority of experimental evidence supporting the association between trace elements, namely additives and contaminants, in food and the GM has been generated in mice models of disease. Yet, mice and humans differ in their microbiota composition, immune function, diets, and metabolism. Thus, interventional studies are also needed. For example, while ethical and logistical concerns require such studies be carefully planned, it should be possible to examine the microbiotas of individuals consuming similar foods that contain, or lack, trace contaminants. Additionally, the use of *in vitro* models of the human gut enables investigating the effects of minor compounds (even those dangerous for humans) without health risks and ethical concerns. Thus, considering the large variety of food additives, trace contaminants and drug residues that can reach consumers, there is a profound need for more in-depth investigations into their effects on the human GM.

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