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14 **Beneficial modulation of the gut microbiota**

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24 **ABSTRACT**

25 The human gut microbiota comprises approximately 100 trillion microbial cells and has a significant  
26 effect on many aspects of human physiology including metabolism, nutrient absorption and immune  
27 function. Disruption of this population has been implicated in many conditions and diseases, including  
28 examples such as obesity, inflammatory bowel disease and colorectal cancer that are highlighted in  
29 this review. A logical extension of these observations suggests that the manipulation of the gut  
30 microbiota can be employed to prevent or treat these conditions. Thus, here we highlight a variety of  
31 options, including the use of changes in diet (including the use of prebiotics), antimicrobial-based  
32 intervention, probiotics and faecal microbiota transplantation, and discuss their relative merits with  
33 respect to modulating the intestinal community in a beneficial way.

34

35 **KEYWORDS**

36 Microbiota; gut health; microbial modulation; faecal microbiota transplant; diet; antimicrobials

## 37 INTRODUCTION

38 Humans are now thought of as “superorganisms” on the basis of the genetic potential encoded within  
39 our resident microbial populations in addition to our own genome. It has been suggested that our  
40 microbiota develops with us and alters its own composition and gene expression in response to  
41 changing environmental conditions [1]. The largest and most varied of the human-associated  
42 microbial communities exists in the gastrointestinal (GI) tract.

43 The gut microbial population is made up of approximately 1000 species from relatively few  
44 phyla. The most abundant species are members of the phyla Firmicutes and Bacteroidetes, with  
45 smaller numbers being representatives of the Proteobacteria, Fusobacteria, Cyanobacteria,  
46 Verrucomicrobia and Actinobacteria, amongst others [2]. The gut microbiota is composed mainly of  
47 anaerobes, which outnumber facultative anaerobes and aerobic bacteria by approximately 2-3 orders  
48 of magnitude [3]. It has been noted that, although there is great inter-individual variation in the  
49 composition of the gut microbiota, there are a conserved set of encoded functions shared between  
50 individuals referred to as the core gut microbiome [4], suggesting that it is the functionality of the  
51 microbiota rather than its composition that is of greatest importance to the host. The functions and  
52 pathways encoded in the core microbiome are thought to confer the greatest benefit to the host and are  
53 probably essential for the correct functioning of the gut. Some well-studied benefits include  
54 protection against potential pathogens, digestion of polysaccharides, production of essential vitamins,  
55 stimulation of angiogenesis, regulation of fat storage and modulation of the host’s immune system [5].  
56 Recent studies have also shown that the gut microbiota influences the gut-brain axis and shapes stress-  
57 related symptoms such as anxiety and pain tolerance [6].

58 Advances in high throughput sequencing technologies (HTS) and tools enabling comparative  
59 analysis of the large amount of data that are generated by these technologies have led to a better  
60 understanding of what constitutes a ‘healthy’ gut microbiota. One of the most interesting observations  
61 drawn from the data generated is that the resident microbiota encodes > 100 fold more genes than the  
62 human genome [7]. The genes present in the microbiome are responsible for many functions essential

63 to host survival but which are not encoded within the human genome. Due to the range and  
64 importance of the metabolic and biochemical processes carried out by the microbiome it has been  
65 referred to as “our hidden organ” [8].

66 While the “healthy” gut microbiota is seen to be a stable community, there are stages within  
67 the life cycle of humans during which there can be dramatic alterations in the structure and function of  
68 this population. These “natural” changes begin with initial colonisation immediately following birth  
69 and subsequent development of the microbiota over the first two years of life. The earliest colonizers  
70 are usually members of the enterococci and enterobacteria followed by strict anaerobes such as  
71 *Bifidobacterium*, *Clostridium* and *Bacteroides* spp. once the initial oxygen supply present has been  
72 depleted [9]. Despite this general pattern, it is important to appreciate that the method of delivery and  
73 subsequent feeding type have a profound effect on the initial populations [10]. Once the infant reaches  
74 two years of age the microbiota has already begun to transform into its adult form, which is thought to  
75 be relatively stable before it undergoes a final “shift” when entering old age [11]. Indeed, with respect  
76 to the latter phenomenon, a study by Claesson and colleagues that compared the gut microbiota of  
77 individuals ages 65 or older to 9 younger control subjects has highlighted significant changes in  
78 community structure associated with ageing, specifically an increase in the abundance of *Bacteroides*  
79 spp. and distinct shifts within the *Clostridium* genus [12]. It has been hypothesised that alterations in  
80 the elderly microbiota are due to physiological changes in the elderly gastrointestinal tract such as  
81 chronic low-grade inflammation, in addition to dietary habits [13].

82 It has been well established that the human gut microbiota is integral to human health, and, as  
83 will be discussed below, it also plays an important role in gastrointestinal disease. It is therefore  
84 reasonable to assume that modulation of the gut microbiota can be used as a therapeutic approach to  
85 treating chronic gastrointestinal diseases. Thus, this review is focussed primarily on the methods that  
86 can be employed to modulate the gut microbiota while highlighting the benefit of guiding community  
87 structure towards a more desirable state.

88

## 89 **ROLE OF THE GUT MICROBIOTA IN GASTROINTESTINAL DISEASE**

90 There are a growing number of gastrointestinal conditions that have been linked with alterations in the  
91 gut microbiota. To properly implement strategies to modulate the gut microbiota as a therapeutic tool,  
92 it is first necessary to understand the role of the gut microbiome in specific GI, and other, diseases.  
93 Given the recent rapid expansion in the number of disease states that have been linked with alterations  
94 in the gut microbiota, it is not possible to address the issue in depth in the confines of this review.  
95 Instead, some well-studied examples are discussed below and we refer you to some other recent  
96 reviews that address this topic in depth [3,14].

### 97 **Inflammatory Bowel Disease**

98 Inflammatory Bowel Disease (IBD) is a relapsing disorder characterized by chronic  
99 inflammation of the GI tract, and of the colon in particular. The two major types of IBD are Crohn's  
100 disease (CD) and ulcerative colitis (UC). Evidence suggests that IBD is a complex disease arising  
101 from a combination of genetic and environmental factors. From a genetics perspective, genome-wide  
102 association studies (GWAS) and subsequent meta-analyses have identified a total of 163 genetic risk  
103 loci for IBD [15-17]. A German twin cohort study confirmed the strong genetic element to IBD by  
104 observing that monozygotic twins are significantly more likely to be concordant for the disease than  
105 dizygotic twins [18]. However, concordance rates between monozygotic twins are nonetheless low  
106 (35% for CD and 16% for UC), highlight that environmental triggers do indeed play an important role  
107 in both diseases, and in UC in particular.

108 It is notable that murine studies have revealed that the presence of commensal enteric bacteria  
109 is necessary for the development of spontaneous colitis and immune system activation [19] and,  
110 indeed, transferring colitogenic gut microbiota into healthy mice can induce spontaneous colitis [20].  
111 Similarly, it has consistently been observed that patients suffering from IBD harbour an altered gut  
112 microbiota [21,22], specifically reduced bacterial diversity and changes within the Firmicutes phylum  
113 [23]. The changes in microbiota composition appear to be somewhat different between UC and CD.  
114 For example, decreased abundance of the butyrate-producing bacteria *Roseburia hominis* and

115 *Faecalibacterium prausnitzii* have been observed in UC patients relative to controls [24], while the  
116 opposite has been observed in CD patients who possessed increased *F. prausnitzii* levels in addition to  
117 a reduced overall diversity [25]. Although these microbial changes could be a result of increased  
118 inflammation, evidence suggests that it is more likely that shifts in the microbiota are involved in the  
119 disease's pathogenesis, either due to an intolerance to a specific group of commensals or due to an  
120 imbalance between protective and harmful members of the population [21,23,26].

## 121 **Irritable Bowel Syndrome**

122 Irritable Bowel Syndrome (IBS) is a chronic GI disorder that presents with symptoms  
123 including abdominal pain, bloating and altered bowel function. IBS is divided into several subtypes  
124 based on stool characteristics; diarrhoea, constipated or mixed. It's cause, as of yet, is not fully known  
125 and although the aetiology is thought to be a combination of a number of factors, it is hypothesised  
126 that perturbations in the normal microbial microbiota play a role in the syndrome's characteristic low-  
127 grade inflammation [27]. Indeed, Rajiić-Stojanović et al. used qPCR and phylogenetic microarrays to  
128 show that the gut microbiota of IBS patients differed significantly from healthy controls, with IBS  
129 sufferers having a 2-fold higher Firmicutes to Bacteroidetes ratio and correlation analysis implicating  
130 several groups of Firmicutes and Proteobacteria in IBS pathogenesis [28]. Contrastingly, Jalanka-  
131 Tuovinen and colleagues observed that the faeces of diarrhoea-predominant IBS sufferers harboured  
132 12-fold higher levels of several Bacteroidetes members. This group also noted that healthy controls  
133 have 35-fold higher numbers of uncultured clostridia [29]. Interestingly, these alterations in the  
134 microbiota correlated with changed in expression of host genes involved in amino acid synthesis, cell  
135 junction integrity and inflammatory response, suggesting impaired epithelial barrier function in IBS  
136 patients. Small intestinal bacterial overgrowth (SIBO), which is characterized by excessive bacteria in  
137 the small intestine, has also been put forward as a possible factor in IBS aetiology [30]. Bacterial  
138 overgrowth can result in overproduction of gas in the small intestine by degradation of carbohydrates,  
139 contributing to the symptoms of IBS [31]. The most commonly isolated bacteria from SIBO patients  
140 are *Escherichia coli*, *Streptococcus*, *Lactobacillus*, *Bacteroides* and *Enterococcus* species [32].  
141 However it is not fully understood if any of these microorganisms play a specific role in IBS

142 progression. It should also be recognised that differences between studies may be due to the causative  
143 microorganisms or imbalances differing between IBS subtypes. Regardless, a bacterial role in IBS  
144 onset would seem to be clear, as further evidenced by the disease's response to antibiotic therapy [33]  
145 and differential expression levels of Toll-like receptors in colonic biopsies of patients with IBS [34].

146

## 147 **Obesity**

148 Obesity is a complex disease resulting from a prolonged imbalance of energy input and energy  
149 expenditure. Modern dietary and exercise habits are major contributing factors but it is now  
150 understood that the composition and function of the gut microbiome plays an important role through a  
151 variety of mechanisms [35]. A number of comprehensive reviews focussing on the association  
152 between the microbiota and obesity have been published [36,37]. Differences in the gut microbiota  
153 between obese and lean individuals have been the subject of great scrutiny. A range of different  
154 murine models have been used to this end, including genetically obese [38,39], diet-induced obese  
155 [40] and humanized [41] mice. Although a number of studies have reported an increased ratio of  
156 Firmicutes to Bacteroidetes in obese mice compared to their lean counterparts, these findings continue  
157 to be the subject of much debate in relation to human studies, which have revealed a number of  
158 microbial populations that have been associated with obesity [37]. Notably, transplanting the faecal  
159 microbiota of obese humans into germ-free mice brought about significant increases in the fat-mass  
160 of, and obesity-related metabolic phenotypes in, these mice relative to those which occurred when the  
161 corresponding faecal microbiota from lean monozygotic twins was transplanted [42]. Furthermore, a  
162 second trial showed that cohousing mice harbouring these two microbial communities prevented  
163 development of the obese phenotype, a trend correlating with invasion of specific Bacteroidetes  
164 members from lean to obese microbiota [42]. Another recent paper of note has linked the mucin-  
165 degrading bacterium *Akkermansia muciniphila* with obesity and type 2 diabetes [43]. The study  
166 showed *A. muciniphila* abundance was decreased in obese and type 2 diabetic mice and that prebiotic  
167 feeding normalised *A. muciniphila* levels, which in turn correlated with an improved metabolic



168 profile. Orally administered *A. muciniphila* also reversed high-fat diet induced metabolic disorders in  
169 these mice [43]. The results of these, and other studies, make it apparent that the microbiota plays a  
170 role in obesity but the specific changes associated with the phenotype are complex and remain  
171 unclear.

## 172 **Type 2 Diabetes**

173 Type 2 diabetes (T2D) is a metabolic disorder with both genetic and environmental  
174 influences. It is a major health concern throughout the western world, arising particularly as a result of  
175 increasing obesity-related insulin resistance [44,45]. It is evident from a number of studies that the gut  
176 microbiome is altered in patients suffering from T2D [46-48], although, as with many obesity-related  
177 associations, it is not clear whether these changes are a cause or simply a consequence of the disorder.  
178 Nonetheless, it was an interesting development when, in 2010 it was reported that the proportions of  
179 Firmicutes, and in particular species of clostridia, were significantly reduced in T2D sufferers  
180 compared to healthy individuals [46]. A subsequent, and much larger, metagenome-wide association  
181 study of 345 Chinese individuals showed that the gut microbiota of patients with T2D was  
182 characterized by a moderate degree of microbial dysbiosis, lower levels of butyrate-producing  
183 bacteria and an enrichment of microbial functions relating to sulphate reduction and resistance to  
184 oxidative stress [48]. Almost all of the microbial genes enriched in T2D patients were from  
185 opportunistic pathogens, including genes from several *Clostridium* spp. as well as *Bacteroides caccae*  
186 [48]. These results provided a number of markers that were assessed to determine if they could  
187 successfully identify patients with T2D on the basis of an analysis of faecal samples. Notably, this  
188 method successfully identified the T2D disease state with 81% accuracy [48], i.e. a greater success  
189 rate than using a combination of clinical risk factors and genetic information [49].

## 190 **Colorectal Cancer**

191 Colorectal cancer (CRC) is the third most common cause of cancer mortality in the world  
192 [50]. It is becoming apparent that, even though a single causative microorganism has not been  
193 explicitly identified, the gut microbiota plays a role in CRC [51,52]. Wang and colleagues noted that

194 there was a clear segregation between the microbiota of CRC patients and healthy volunteers,  
195 particularly, as was the case for T2D, a decrease in the abundance of butyrate producers and an  
196 increase in the incidence of opportunistic pathogens in CRC patients [53]. Members of the  
197 *Fusobacterium* genus have also been recently identified as potential causative agents after it was  
198 observed that they were enriched in colorectal carcinomas [54], a pattern also noted in other studies  
199 [53,55-57]. The authors hypothesised that *Fusobacterium* spp. may contribute to tumourigenesis by an  
200 inflammatory-mediated mechanism, a hypothesis supported by a follow-up study which showed that  
201 members of fusobacteria could generate a proinflammatory microenvironment through the  
202 recruitment of tumour-infiltrating immune cells [58]. *E. coli* has also been linked with CRC in a  
203 number of studies. Arthur *et al.* observed that *E. coli* levels were ~100-fold higher in the microbiota  
204 of the colitis-susceptible *IL10*<sup>-/-</sup> mouse strain compared to the wild type [51]. They went on to show  
205 that *E. coli* NC101 mono-association significantly promoted development of invasive mucinous  
206 adenocarcinomas in azoxymethane treated, *IL10*<sup>-/-</sup> mice and that deletion of the polyketide synthase  
207 (*pks*) genotoxic island from this *E. coli* strain decreased tumour multiplicity and invasion [51]. While  
208 further investigations are required, these results suggest that colitis promotes tumourigenesis in mice  
209 by altering the composition of the gut microbiota and selecting for members with genotoxic  
210 capabilities.

211 Ultimately, identification of microorganisms, microbial populations or microbial  
212 functionalities involved in GI disease is fundamental to developing novel therapies. It is evident that  
213 the gut microbiota plays a large role in intestinal health and disease, and therefore manipulation or  
214 modulation of this community, is a clinical option that merits serious consideration.

215

## 216 **MODULATION OF THE GUT MICROBIOTA**

### 217 **Modulation by Diet**

218 Environmental factors, including dietary intake, can shape the composition of the intestinal  
219 microbial community. Indeed, a number of recent studies have highlighted the links between diet and  
220 distinct microbial profiles and, in turn, overall gut health [40,59-63]. Having an understanding of how  
221 diet influences microbial communities will be of critical importance with respect to employing food to  
222 beneficially alter the gut microbiota.

223 The amount, type and balance of the three main dietary components, i.e. protein,  
224 carbohydrates and fat, have a profound impact on the gut microbiota. Short-chain fatty acids (SCFAs),  
225 primarily butyrate, propionate and acetate, are the major end products from the microbial degradation  
226 of carbohydrates and protein in the gut. SCFAs have a diverse range of physiological effects on the  
227 host, with perhaps the most important being their oxidation by mucosal cells to provide energy. An  
228 excellent review of the benefits of SCFAs on the host has been published by Macfarlane &  
229 Macfarlane [64]. The majority of microbial protein degradation occurs in the distal colon where the  
230 pH is neutral and conditions are favourable for the growth of proteolytic bacteria such as *Bacteroides*  
231 spp., *Propionibacterium* spp. and *Clostridium perfringens* [65,66]. The main pathway of protein  
232 degradation by this population is deamination of amino acids to the aforementioned SCFAs and  
233 ammonia [67], high concentrations of the latter have been shown to act as tumour promoters in rats  
234 [68]. The range of end products generated by protein digestion is broader than that of carbohydrates  
235 (see below) and also includes branched-chain amino acids, phenols, indoles and amines [69]. The  
236 majority of studies examining the effect of dietary protein on the gut microbiota have focussed  
237 primarily on the detection of altered fermentation products in the cecum [70] and faeces [71].  
238 However, the effects of whey protein isolate on the microbiota have been the topic of some scrutiny in  
239 recent years as it has been indicated that dairy products can alleviate several disorders relating to  
240 metabolic syndrome [72]. One such study noted significantly increased counts of bifidobacteria and  
241 lactobacilli in the faeces of rats whose diets included cheese whey protein isolate or casein  
242 supplemented with either threonine or cysteine [73]. Whey protein isolate (WPI) has also been  
243 observed to alter the composition of the gut microbiota of mice in a dose-dependent manner [74]. All  
244 mice whose high fat diet was supplemented with WPI had significantly increased proportions of

245 *Lactobacillaceae* and significantly decreased proportions of *Clostridiaceae* compared to high-fat fed  
246 controls, and increasing the amount of total energy derived from WPI caused a more profound shift in  
247 the microbiota [74]. Certain components of the normal human dietary intake of carbohydrates cannot  
248 be digested directly by the host and act as the major diet-derived energy source for microorganisms in  
249 the gut [75]. This fraction, comprised largely of resistant starches and non-starch polysaccharides, is  
250 degraded by microbial fermentation to a mixture of gasses and the aforementioned SCFAs. Many  
251 such carbohydrates are also referred to as prebiotics. The term prebiotic was introduced by Gibson  
252 and Roberfroid in 1995 [76] and are defined as “selectively fermented ingredients that allow specific  
253 changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits  
254 upon host well-being and health” [77]. Prebiotics have most frequently been employed with a view to  
255 stimulating the growth of either lactobacilli or bifidobacteria, with many studies focussing on inulin  
256 [78-80], oligofructose [81,82] or fructooligosaccharides [83,84]. There is a substantial body of  
257 evidence linking prebiotic consumption to human health benefits through modulation of the gut  
258 microbiota, with research in this area having been the subject of a number of recent reviews [85-87].  
259 In one particularly notable recent study, it was observed that supplementing the murine diet with  
260 SCFAs or fructooligosaccharides caused a shift in microbiota composition which strongly correlated  
261 with beneficial changes in body weight, adiposity and glucose control. These physiological changes  
262 were brought about via butyrate- and propionate-mediated activation of intestinal gluconeogenesis  
263 [88].

264 The majority of dietary fat is absorbed in the human small intestine but it has been shown that  
265 a substantial amount survives digestion and can be recovered in faeces [89]. The undigested portion  
266 passes through the colon where it can have a profound effect on the intestinal microbiota. Murphy *et*  
267 *al.* observed that high-fat feeding caused a greater compositional change in the gut microbiota than  
268 genetically induced obesity [90], in accordance with a previous study which showed that, when fed a  
269 high-fat diet, RELM $\beta$  knockout mice showed a significantly altered gut community while staying  
270 lean. RELM $\beta$  knockout mice were employed as they are known to stay relatively lean when fed a  
271 high-fat diet. The authors could therefore conclude that the change in diet, as opposed to the obese

272 state, was responsible for the observed changes in the microbiota [91]. Many studies have established  
273 that mice fed a high-fat diet have significantly dissimilar microbial populations in the gut compared to  
274 mice fed on normal chow [38,40,92]. However, a recently published study showed that life-long  
275 calorie restriction significantly altered the gut microbiota in mice fed on both high-fat and low-fat  
276 diets [93]. This implies that not only the fat content of the diet, but also the number of calories  
277 consumed, has the potential to influence the bacterial communities present in the GI tract. The study  
278 also linked changes in the gut microbiota to claims that calorie restriction promotes healthy-ageing  
279 and increases lifespan in various animal models as the healthiest and longest living mice were those  
280 that were fed a low fat diet with calorie restriction [93]. In addition to the studies referenced above,  
281 there are many excellent reviews of the effect of dietary fat on the intestinal microbiota [37,94,95].

282         This specific combination of dietary components can vary according to geographic location,  
283 food availability, cultural practices and age and can have a profound impact on the conditions within  
284 the gut and the requirements of the microbiota (Table 1 highlights some studies which have  
285 investigated this impact). In one instance, the faecal microbiota of European children and children  
286 from an African village in Burkina Faso, whose diets differed considerably, was investigated. The diet  
287 of the African children was predominately vegetarian; high in starch, fibre and plant polysaccharides  
288 and low in fat and animal protein. This diet correlated with a significant increase in the  
289 Bacteroidetes:Firmicutes ratio in addition to an abundance of *Prevotella* and *Xylanibacter* when  
290 compared to the microbiota of the children consuming a carbohydrate-rich European diet [96]. The  
291 *Xylanibacter* genus, which was absent in European children, is known to contain genes for xylan and  
292 cellulose hydrolysis and so it was hypothesised that the gut microbiota coevolved with the  
293 polysaccharide-rich diet of the Burkina Faso children, allowing them to increase the energy extracted  
294 from dietary fibre while also conferring protection from inflammation and non-infectious colonic  
295 disease [96]. The comparatively high abundance of *Prevotella* in the faecal microbiota of the African  
296 children and the fact that it coincides with a carbohydrate-rich diet is consistent with the observations  
297 of Wu *et al.* who found that the overall composition of the microbiota was strongly associated with  
298 long-term diet [62]. Specifically, a diet rich in protein and animal fat was associated with higher

299 proportions of *Bacteroides* while *Prevotella* were more abundant when the diet was enriched with  
300 plant-derived carbohydrates [62]. A recent study by De Filippo *et al.* took these investigations a step  
301 further by focussing specifically on the effect of diets composed entirely of animal or plants products  
302 on the gut microbiota [61]. It revealed that an animal-based diet increased the numbers of bile-tolerant  
303 microorganisms present and decreased the numbers of plant polysaccharide degrading Firmicutes.  
304 Interestingly, the respective diets brought about a transcriptional response among the gut microbiota  
305 that was consistent with previously reported differences in gene abundances between herbivorous and  
306 carnivorous animals [61]. In other studies, members of the *Clostridium* clusters IV and XIVa have  
307 been found to be enriched in the faeces of omnivores compared to vegetarians and lacto-vegetarians,  
308 who generally consume higher proportions of carbohydrates as part of their diet [97-99]. These  
309 clusters of bacteria are noted for their ability to convert dietary fibre to SCFAs.

310         The overall dietary patterns in the De Filippo study above are similar to a study in mice where  
311 conventionalised mice were switched from a low-fat diet rich in complex plant polysaccharides  
312 (CHO) to an obesity-inducing high-fat/simple carbohydrate “Western” diet [40]. Mice fed on the  
313 “Western” diet had a significantly lower level of bacterial diversity, a characteristic seen to be an  
314 indicator of an unhealthy microbiota [59]. These mice possessed a significantly higher relative  
315 proportion of Firmicutes and lower relative proportions of Bacteroidetes compared to littermates  
316 which remained on the CHO diet. This population shift is similar to what is seen in the *ob/ob* mouse  
317 model of obesity [38] but differs in that the Firmicutes shift in the genetically-induced obesity model  
318 is division-wide whereas the dietary intervention above caused a bloom in a single uncultured clade  
319 within the Mollicutes class. A subsequent microbiota transplantation from these diet-induced obese  
320 mice into germ-free recipients promoted greater adiposity than transplants from lean donor [38]. A  
321 further study by the same group showed that this response of the microbiome to dietary intervention is  
322 rapid and can occur within 24 hours [41], a phenomenon also observed by Wu *et al.*, [62].

323         A gut microbiota with decreased diversity has been linked with increased frailty and poorer  
324 general health in elderly subjects [60]. In this study, clustering of subjects by diet, residence location  
325 and by microbial groupings was apparent. Ultimately, it was evident that subjects that were living in

326 the community had a healthier and more varied diet than subjects in long-term residential care, which  
327 gave rise to a more diverse gut microbiota with significant changes being noted at phylum and family  
328 levels. Differences were also apparent at the genus level with long-stay subjects possessing higher  
329 levels of *Parabacteroides*, *Eubacterium*, *Anaerotructus*, *Lactonifactor* and *Coprobacillus*, while  
330 *Coprococcus* and *Roseburia* (both members of the *Lachnospiraceae* family) were more abundant in  
331 community-dwelling subjects [60]. The data also linked microbiota composition to the duration spent  
332 in long-stay care. The longer the subject stayed in residential care (and consumed a less varied diet),  
333 the more dissimilar their microbiota became to the microbiota of healthy community-dwelling  
334 subjects [60]. Another study investigating the temporal relationship between food intake, gut  
335 microbiota and metabolic and inflammatory phenotypes reported that individuals with reduced  
336 microbial gene richness present more pronounced dys-metabolism and low-grade inflammation than  
337 their richer counterparts [100]. This microbiota-associated phenotype was suggested to be a result of  
338 long-term dietary habits as it was noted that these subjects seemed to consume less fruits, vegetables  
339 and fish than their high gene richness equivalents, i.e. a pattern consistent with that reported by  
340 Claesson *et al* [60]. More specifically, the initial sampling of the cohort (49 obese or overweight  
341 subjects) showed that subjects with lower gene richness in the gut microbiota presented with  
342 increased obesity-associated phenotypes such as higher insulin resistance and increased levels of  
343 fasting serum triglyceride, LDL cholesterol and inflammation. Dietary intervention (6 week energy-  
344 restricted high-protein diet) increased gene richness significantly in individuals that originally had a  
345 low gene count. This increased gene richness remained after the subjects were switched to a 6 week  
346 weight-maintenance diet suggesting that dietary intervention as the potential to, at least partially,  
347 correct a loss of richness in the microbiota [100].

348         Given the complexity of the relationship between diet and the gut microbiota, there would  
349 seem to be merit in developing and utilising models that allow one to elucidate the specific  
350 relationship between specific dietary components and microorganisms. An elegant strategy to  
351 facilitate this was provided by Faith *et al.* when they introduced a model community of ten human gut  
352 bacteria into gnotobiotic mice and developed a relatively simple statistical model which predicted

353 over 60% of the species variations that occurred in response to changes in diet [101]. The amount of  
354 casein in the diet was observed to be significantly associated with the abundances of all 10 microbial  
355 species and highly correlated with the total biomass of the community. Interestingly, *E. coli* and  
356 *Clostridium symbiosum* were the only two species that had a second dietary variable significantly  
357 associated with their abundance, sucrose and starch respectively. The statistical model was  
358 subsequently able to determine 61% of the variation of the community members when the host was  
359 fed a new, previously unseen diet [101]. These results represent a significant step towards tailoring  
360 diet to address chronic microbiota-associated illnesses and a potential evolution of research within the  
361 field.

362 It is clear that microbial composition varies between groups living on different long-term  
363 diets. Recent investigations that suggest that short-term dietary changes can also alter the  
364 composition, and result in changes to the metabolic activity of the microbiome as a whole, are  
365 noteworthy but further investigations are required to determine how best to take advantage of these  
366 observations.

### 367 **Modulation by Antimicrobials**

368 The manipulation of the gut microbiota by antimicrobials is emerging as an attractive  
369 therapeutic strategy (Table 2). The success of this approach is likely to ultimately depend on the target  
370 specificity of the antimicrobials in question, especially as the undesirable consequences of the overuse  
371 of broad-spectrum antimicrobials have become ever more apparent in recent years. For quite some  
372 time broad-spectrum antibiotics have been commonly used by clinicians as they can be used in the  
373 treatment of a wide range of infections or when the causative bacterium has not been formally  
374 identified. However, due to the frequent use of these antibiotics, the spread of antibiotic resistance is  
375 now posing a serious problem in health care settings. In addition, antibiotic therapies not only affect  
376 the target microorganism but can also perturb the host gut microbial communities. The extent of this  
377 damage has recently become more evident through the application of high throughput DNA-based  
378 sequencing technologies to assess the composition of gut microbial populations (for review see Cotter



379 et al. 2012) [102]. Here we provide just a few examples of the negative consequences of the use of  
380 broad-spectrum antibiotics on the gut microbiota and, in turn, health.

381         The widespread use of broad-spectrum antibiotics, such as amoxicillin, to treat childhood  
382 infections has been linked to a dramatic decrease in *Helicobacter pylori* carriage [103]. However,  
383 studies indicate that those who did not acquire *H. pylori* in childhood were more likely to  
384 subsequently develop asthma, hay fever and skin allergies [104], while other investigations suggest  
385 that *H. pylori* infection has a protective effect with respect to the development of allergic asthma in  
386 mouse models [105]. The use of some broad-spectrum antibiotics, including clindamycin, ampicillin,  
387 amoxicillin, cephalosporins and flouroquinolones, can also result in *Clostridium difficile* overgrowth  
388 by impacting the resident gut microbiota, followed by antibiotic-associated diarrhoea,  
389 pseudomembranous colitis and, potentially, life-threatening complications such as toxic megacolon  
390 [106,107]. Low doses of antibiotics have also been used as growth promoters in agriculture since the  
391 1950's despite an unclear understanding of the mechanisms at work. A recent investigation into this  
392 effect revealed subtherapeutic antibiotic treatment (STAT) of various antibiotics increased adiposity  
393 and hormones related to metabolism in young mice compared to untreated controls [108]. Analysis of  
394 the composition and function of the gut microbiota of these animals made it apparent that STAT  
395 exposure selected for microbial species that were able to extract more calories from dietary complex  
396 carbohydrates that were otherwise indigestible in the control group [108].

397         When considering these results, it is important to be aware that different broad-spectrum  
398 antibiotics differ with respect to their impact on the gut microbiota. Changes to the gut microbiota can  
399 also be either long- or short-term. In one instance this was highlighted through murine studies which  
400 established that mice treated with a cocktail of amoxicillin, metronidazole and bismuth [3.0 mg, 0.69  
401 mg and 0.185 mg, respectively] daily for 10 days had largely recovered their baseline microbial  
402 community structure 2 weeks post-treatment but that treatment with cefoperazone [0.5 mg/ml of  
403 drinking water] had long-term effects on community structure and reduced overall diversity [109].  
404 The effect of an antibiotic on the gut microbiota is influenced by several factors including its

405 antimicrobial effect (bactericidal or bacteriostatic), its mode of action, the structure of the microbiota  
406 and the distribution of antibiotic resistance genes among this population [110].

407         In light of this greater appreciation of the impact of broad spectrum antimicrobials on the gut  
408 microbiota, it is apparent that there is value in utilising antimicrobials with a narrow spectrum of  
409 inhibition. In addition to existing repositories of narrow spectrum antimicrobials that were not  
410 previously commercialised, it is worth noting that the gut microbiota is considered a rich, but yet  
411 relatively, underutilised source of antimicrobial-producing, and in particular bacteriocin-producing,  
412 bacteria. Bacteriocins are ribosomally synthesised peptides to which the producer has a specific  
413 immunity gene and can have either a narrow or broad spectrum of activity [111]. Many bacteriocins  
414 have a number of desirable traits, including low toxicity, high potency and, in the case of gut  
415 associated strains, the possibility of *in situ* antimicrobial. This combination of traits makes them  
416 attractive alternatives to traditional antibiotic therapies. Despite being, as stated above, a relatively  
417 underutilised source of antimicrobials, a number of bacteriocins have previously been isolated from  
418 mammalian gut microbes [112-115]. Indeed, for example, screening of faecal samples from 266  
419 elderly Irish subjects identified 13 bacteriocin producing strains [115] while a further study lead to the  
420 isolation of 23 distinct bacteriocin-producing strains from a range of mammalian gastrointestinal  
421 sources [112]. Given that, for a bacteriocin to be produced and be active in the gut, the producer needs  
422 to be able to survive in and colonize the human gut and the associated antimicrobial needs to be active  
423 in the gut environment, it has been argued that the gut is an ideal source of bacteriocin producers with  
424 the potential to alter the gut microbiota [116]. There have already been a number of studies which  
425 have highlighted the merits of employing gut-associated bacteriocins, several of which we refer to  
426 here. In a distal colon model, the narrow spectrum bacteriocin thuricin CD has been observed to  
427 inhibit the growth of *C. difficile* without having any significant additional impact on the other  
428 components of the gut microbiota [113]. This contrasted with the significant shift in the relative  
429 proportions of the dominant bacterial populations that were observed when the broad-spectrum  
430 antimicrobials lacticin 3147, metronidazole and vancomycin, respectively, were employed. Notably,  
431 thuricin CD also exhibited a potency comparable to that of the control antimicrobials [113], thereby

432 establishing that thuricin CD has potential as an alternative to the conventional antimicrobial  
433 strategies employed to treat *C. difficile* infection, especially as it is less likely to impact negatively on  
434 the commensal gut microbiota and, thus, is more likely to prevent recurrent *C. difficile* infections.  
435 While, in the above example, thuricin CD, rather than the associated *Bacillus thuringiensis* producer  
436 [106], was employed, there are other examples that have highlighted the merits of using the  
437 bacteriocin-producing strain itself. In one such instance, ingestion of the bacteriocin producing  
438 probiotic strain *Lactobacillus salivarius* UCC118 provided significant protection against infection by  
439 *Listeria monocytogenes* in mice [117]. Production of the Abp118 bacteriocin by UCC118, which has  
440 previously been shown to be capable of altering the intestinal microbiota of pigs and mice [118],  
441 proved to be the key protective factor as a non-bacteriocin producing mutant failed to confer the same  
442 protection. This protective effect was also lost when infection was with a bacteriocin-immune *L.*  
443 *monocytogenes* mutant, thereby confirming that the mode of action was direct antagonism by Abp118  
444 rather than *via* some other indirect effect [117]. In another instance a combination of 5 probiotic  
445 strains were employed to control *Salmonella* Typhimurium-induced diarrhoea in pigs [119]. It was  
446 subsequently established that the only bacteriocin-producing strain, *L. salivarius* DPC6005, was the  
447 dominant member of the cocktail in both the ileum digesta and in the mucosa. It could not be  
448 established, however, if bacteriocin production was directly responsible for anti-*Salmonella* activity  
449 [120].

450 In addition to the control of pathogens, antimicrobials have also been investigated with a view  
451 to altering metabolic health in diet-induced obese mice [121]. Supplementation of a high-fat diet with  
452 vancomycin caused a significant decrease in Firmicutes and Bacteroidetes populations with a  
453 corresponding increase in Proteobacteria. This compositional shift was accompanied by a marked  
454 decrease in weight gain, fasting blood glucose, plasma TNF $\alpha$  and triglyceride levels compared to the  
455 diet-induced obese controls. Although supplementation of the high-fat diet with the bacteriocin-  
456 producing probiotic *L. salivarius* UCC118 did not produce any significant changes in the metabolic  
457 profiles of the mice, it did result in an increase in relative proportions of Bacteroidetes and  
458 Proteobacteria with a corresponding decrease in Actinobacteria. The authors concluded that

459 antimicrobial strategies have the potential to alter both the composition of the gut microbiota and the  
460 metabolic health of the host. However, it was noted that care must be taken when choosing the  
461 antimicrobial to be used so as to bring about extended beneficial impacts on metabolic health.

462 As with diet, the vast majority of work concerning modulation of the microbiota by  
463 antimicrobials has taken place in mouse models. Nevertheless, the results are encouraging and suggest  
464 that carefully selected antimicrobials represent a viable option with respect to intelligently altering the  
465 bacterial populations within the human gut.

### 466 **Modulation by Probiotics**

467 The World Health Organization defines probiotics as “live microorganisms which when administered  
468 in adequate amounts confer a health benefit on the host” [122]. Probiotics are becoming increasingly  
469 popular and are generally marketed as functional foods or dietary supplements. As it has been  
470 recognised that changes in the gut microbiota play a role in GI disease then it is not surprising that  
471 probiotics are an attractive option with respect to modulation of the gut microbiome. For a probiotic to  
472 successfully exert its benefit on the host’s gut microbiota it should be able to remain viable during  
473 storage and also be capable of surviving, and potentially colonizing, the host’s intestinal environment  
474 [123]. The majority of probiotics currently used are members of lactic acid bacteria (LAB) and, more  
475 specifically, strains from the genera *Lactobacillus* and *Bifidobacterium* are most commonly used in  
476 commercial probiotics. Mixtures of these strains are becoming increasingly popular as researchers  
477 gain a deeper understanding of increasing efficacy via possible additive or synergistic effects [124].  
478 Rijkers *et al.* categorised the benefit of probiotics into three levels based on location and method; 1)  
479 interference with the growth or survival of pathogenic microorganisms in the gut lumen, 2)  
480 improvement of mucosal barrier function or mucosal immune system and 3) influence beyond the gut  
481 through the systemic immune system and other organs [125]. A study undertaken by Park *et al.* found  
482 that DIO mice treated with the probiotic strains *Lactobacillus curvatus* HY7601 and *Lactobacillus*  
483 *plantarum* KY1032 experienced reduced body weight gain and fat accumulation in addition to  
484 lowered plasma insulin, leptin, total-cholesterol and liver toxicity biomarkers compared to a group on

485 the same diet supplemented with a placebo [126]. Supplementation with these probiotic strains also  
486 resulted in down-regulation of pro-inflammatory genes in adipose tissue, up-regulation of fatty acid  
487 oxidation-related genes in the liver and significant alterations in the diversity and function of the gut  
488 microbiota. Similar results were observed by Yadav *et al.*, who found that administration of the  
489 probiotic VSL#3 prevented and treated obesity and diabetes in a number of different murine models  
490 through modulation of the gut microbiota. In particular, an increase in the number of butyrate-  
491 producing bacteria was linked with enhanced secretion of the hunger-reducing hormone GLP-1 as  
492 well as upregulation of genes involved in GLP-1 synthesis and excretion [127]. McNulty *et al.*  
493 observed that, in gnotobiotic mice harbouring a 15-member model human gut microbial community,  
494 introduction of 5 probiotic strains isolated from a fermented milk product did not significantly alter  
495 the composition of the intestinal microbiota but instead increased the expression of microbial genes  
496 involved in carbohydrate and nucleotide metabolism while decreasing expression of genes involved in  
497 the metabolism of lipids and amino acids [128]. These metatranscriptomic changes were also apparent  
498 in the microbiota of human monozygotic twins when fed the same fermented milk product, primarily  
499 upregulation of genes involved in carbohydrate metabolism. In addition to their investigation with a  
500 view to contributing to the prevention/treatment of obesity and T2D, it should be noted that probiotics  
501 are thought to have the potential to treat a wide range of other conditions such as IBS, allergies, *C.*  
502 *difficile* infection, IBD and others by modulation of the gut microbiota as highlighted in a number of  
503 recent manuscripts [129-135]. As we learn more about other gut microbes and their role in human  
504 health it may emerge that the future of probiotics lies in different, non-traditional probiotics, for  
505 example *Akkermansia muciniphila* as mentioned previously [43]. A recent review by Neef and Sanz  
506 discusses some of the strains already being investigated and the new techniques employed to assess  
507 their impact on human health [136].

### 508 **Modulation by Faecal Microbiota Transplantation**

509 Following on from the probiotics principle, but on a community rather than strain level, faecal  
510 microbial transplantation (FMT) is the process of transplanting faecal bacterial communities from a  
511 healthy individual to a recipient whose microbiota has been disrupted or altered. Although still

512 somewhat in its infancy, FMT is becoming more commonly used as an approach to replenish the gut  
513 microbiota in order to alleviate the symptoms of disease. To date, FMT has most commonly been  
514 used to treat recurrent *C. difficile* infection (CDI) by replacing populations of commensal bacteria  
515 which have been wiped out by antibiotic therapy. Khoruts and colleagues used terminal-restriction  
516 fragment length polymorphism and 16S rRNA approaches to compare the bacterial component of a  
517 CDI patient's microbiota before and after FMT intervention [137] and found that, before intervention,  
518 the microbiota was deficient in both Bacteroidetes and Firmicutes but 14 days post-transplantation the  
519 microbiota was changed to closely resemble the donor's microbiota and was dominated by  
520 *Bacteroides* spp. [137]. These results are similar to findings by Tvede and Rask-Madsen who reported  
521 *Bacteroides* spp. were absent in CDI patients but were replenished after FMT intervention [138]. The  
522 composition of the donor's microbiota is the key factor in determining the efficacy of this treatment,  
523 as shown by Grehan et al. who collected faecal samples from patients undergoing FMT at 4 time  
524 points; pre-treatment and at intervals of 4, 8 and 24 weeks post-treatment to determine the effect of  
525 FMT on its microbial content [138]. Using a molecular approach they found that the microbiota was  
526 altered by FMT intervention and that at 4, 8 and 24 weeks the community of the recipient was  
527 composed predominately of bacteria derived from the healthy donor's samples. Crucially, in addition  
528 to bringing about desirable microbiota-related changes, FMT has in a high frequency of cases been  
529 successful in controlling CDI. In one such study it was revealed that only 1 of 16 patients treated with  
530 FMT experienced a recurrence of colitis during the 90 day follow-up period [139]. Indeed, when  
531 many such studies were combined in a systematic literature review by Gough *et al.*, i.e. to examine  
532 the effect of FMT on 317 CDI patients across 27 case studies, it was revealed that disease was  
533 resolved in 92% of cases [140]. An interesting development in the application of FMT is the use of  
534 synthetic microbial communities in place of undefined mixtures from donors (for review see de Vos et  
535 al [141]). The synthetic mixtures have the advantage of being controlled, tested extensively for the  
536 presence of viruses or pathogens and have the potential to be reproducibly manufactured. Petrof *et al.*  
537 showed that a defined mixture of 33 isolates, when administered during a colonoscopy, cured the CDI  
538 of 2 patients who had previously failed to respond to antibiotic treatment [142]. 16S rRNA analysis  
539 showed that the strains found in the stool substitute were rare in the patient's gut microbiota before

540 intervention, however following treatment these strains accounted for over 25% of sequences  
541 recovered from the gut microbiota. Although FMT has been most extensively studied with a  
542 view to CDI treatment, it has, however, also been investigated as a potential treatment option for a  
543 range of microbiota-associated diseases including IBD, IBS, obesity, idiopathic thrombocytopenic  
544 purpura and even multiple sclerosis. A recently published review by Borody *et al.* summarises the  
545 current state of research and possible future directions of the technique [143].

## 546 **CONCLUDING REMARKS**

547 It is well established that the gut microbiota influences host metabolism, nutrient absorption  
548 and immune function, and that disruption of this balanced community can have very serious health  
549 implications. As we gain a deeper understanding of the specific relationships between the gut  
550 microbiota and disease, we expose potential therapeutic targets. Intelligent modulation of the  
551 intestinal community is a topic that has gained considerable interest and has the possibility to be  
552 extremely beneficial for human health.

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## 555 **CONFLICT OF INTEREST STATEMENT**

556 The authors declare no conflicts of interest in preparing this article.

557

558 Table 1. Some examples of studies assessing the influence of diet on the microbiota and health of the  
 559 host.

<b>Diet</b>	<b>Effect on microbiota</b>	<b>Effect on host</b>
Rich in plant-derived polysaccharides [62,96].	Increased Bacteroidetes, decreased Firmicutes [96]. Associated with <i>Prevotella</i> -rich enterotype [62].	Faster gut transit time compared to high protein and animal fat diet [62].
Omnivorous compared to vegetarian and lacto-vegetarian [97-99].	Increased <i>Clostridium</i> clusters IV and XIVa [97-99].	Not reported
High-fat, simple carbohydrate “Western” diet [38,40].	Increased Firmicutes, decreased Bacteroidetes [38,40].	Diet-induced obesity. Subsequent transplantation of obese microbiota to germ free mice increased adiposity [40].
Reduced carbohydrate intake [63].	Reduced <i>Bifidobacterium</i> , <i>Roseburia</i> spp. and <i>Eubacterium rectale</i> [63].	Not reported
Animal product-based [61]. High protein and animal fat [62].	Increased $\beta$ -diversity and bile-tolerant bacteria, including <i>Bacteroides</i> , decreased Firmicutes [61]. Associated with <i>Bacteroides</i> -rich enterotype [62].	Decreased weight independent of calories consumed [61].
Less fruit, vegetables and fish [100].	Reduced microbial gene richness [100].	Increased insulin resistance, fasting serum triglyceride levels, LDL cholesterol and inflammation [100].
Reduced variety due to long-stay care [60].	Increased Bacteroidetes and reduced overall diversity [60].	Increased frailty and poorer general health [60].
Changed from a vegetarian diet to an animal-based diet [61].	Decreased <i>Prevotella</i> , increased <i>Bacteroides</i> [61].	Not reported

560

561



562 Table 2. Some examples of studies assessing the influence of antimicrobials on the gut microbiota  
 563 and, where relevant, the host.

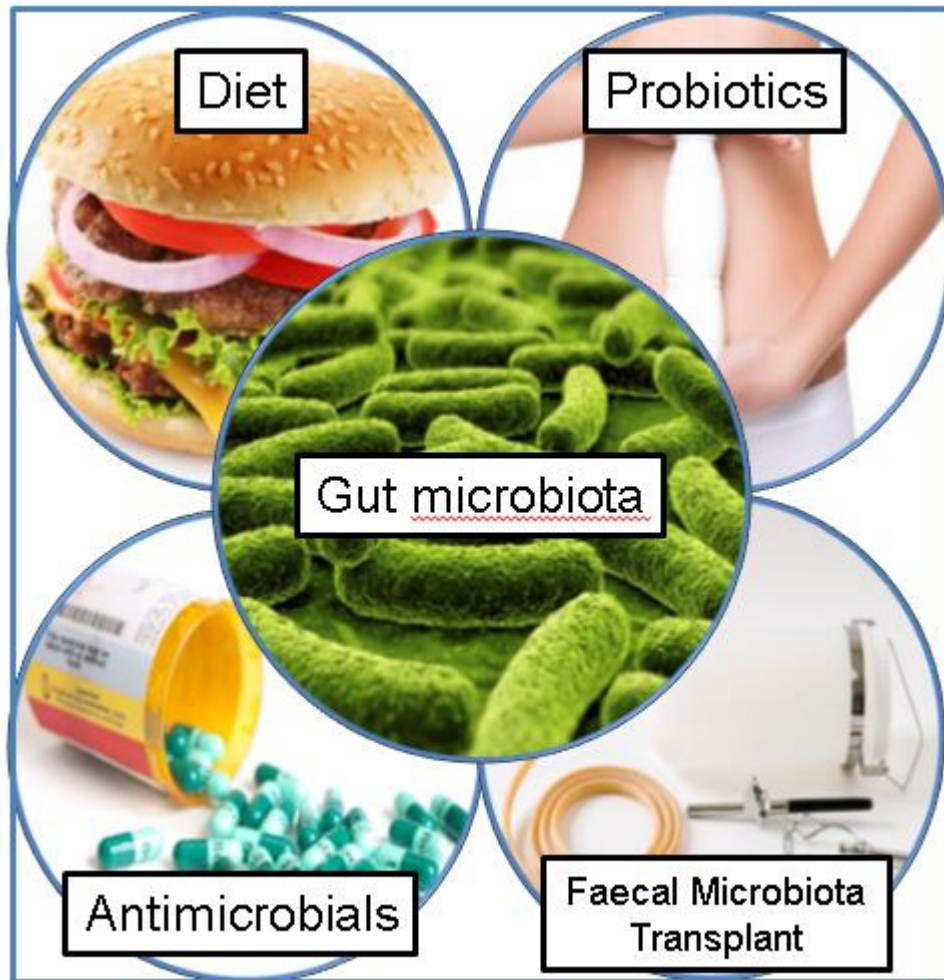
Antimicrobial	Effect on Microbiota	Physiological effect on host
Thuricin CD	Eliminated <i>C. difficile</i> without impacting overall microbiota composition [113].	Not examined – distal colon model
Abp118	Protection against <i>Listeria monocytogenes</i> infection [117]. Increased Bacteroidetes and Proteobacteria, decreased Actinobacteria [120].	Temporarily reduced weight gain in pigs [117].
Vancomycin	Decreased Firmicutes and Bacteroidetes, increased Proteobacteria [121].	Decrease in weight gain, fasting blood glucose, plasma TNF $\alpha$ and triglyceride levels in DIO mice [121].
Sub-therapeutic antibiotic therapy*	Increased Firmicutes, especially <i>Lachnospiraceae</i> , relative to Bacteroidetes [108].	Increased adiposity and bone mineral density in mice [108].
5 strain probiotic mixture**	Reduced shedding of <i>Samonella enterica</i> serovar Typhimurium in pigs[119].	Reduced incidence, severity and duration of diarrhoea in pigs. Also, increased weight gain [119].
<i>Lactobacillus gasseri</i> SBT2055, producer of gassericin T bacteriocin	Not reported	Decreased abdominal adiposity, body weight, BMI, waist circumference and hip circumference in human adults [131]. Lower triglyceride levels and reduced expression of lipogenic and pro-inflammatory genes in DIO mice [135].

564 \* Penicillin, vancomycin, penicillin plus vancomycin, and chlortetracycline

565 \*\* *Lactobacillus murinus* DPC6002, *Lactobacillus murinus* DPC6003, *Lactobacillus*

566 *pentosus* DPC6004, *Lactobacillus salivarius* DPC6005, and *Pediococcus pentosaceus* DPC6006

567



568

569 Fig. 1. Potential strategies for manipulation of the gut microbiota.

570

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