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Molecular mechanisms of probiotic prevention of antibiotic-associated diarrhea

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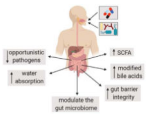
Abstract

Antibiotic-associated diarrhea (AAD) is a common and unintended adverse effect of antibiotic treatment. It is characterized by the disruption of the gut microbiota, decreased intestinal short chain fatty acid (SCFA) concentrations, accumulation of luminal carbohydrates and colonic bile acids, altered water absorption, and ultimately diarrhea. Probiotics were shown to prevent AAD in numerous clinical trials. This review examines what is currently known about how probiotics reduce the risk for AAD via modulating the gut microbiota, altering nutrient and bile acid metabolism, inducing epithelial solute transporter activity, supporting intestinal barrier function, and influencing the immune system. Although probiotics are frequently prescribed with antibiotic use, mechanistic evidence verifying how they confer protection against AAD is extremely limited. This information is urgently needed for improving recommendations for sustaining probiotic development and for implementing probiotics in clinical settings.

Graphical Abstract



Keywords

antibiotics; gut microbiome; probiotic; Lactobacillus; Bifidobacterium; diarrhea; AAD; SCFA

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Solomon A. Mekonnen: Writing - original and revised draft preparation. Daniel Merenstein: Reviewing and editing. Claire M. Fraser: Reviewing and editing. Maria L Marco: Conceptualization, supervision, writing - original and revised draft preparation, review and editing.

Declaration of interest

None

Introduction

Antibiotic-associated diarrhea (AAD) is an important morbidity resulting from antibiotic use. AAD is more than a bothersome adverse event of antibiotic treatment; it is associated with prescription noncompliance and overuse of second-line antibiotics. Although all age groups are affected by AAD, children are particularly at risk because they are often placed on antibiotics, and the rate of diarrhea associated with antibiotic usage among children is between 20 to 35% [1]. AAD is defined as clinically unexplained diarrhea that occurs in connection with antibiotic administration. Any antibiotic could potentially cause AAD, but broad-spectrum antibiotics that predominantly target anaerobes and are poorly absorbed (such as clindamycin, cephalosporins (cefixime and ceftriaxone), and amoxicillin-clavulanate) have a higher AAD incidence [2].

One of the most commonly prescribed uses of probiotics is for the prevention of antibiotic-associated diarrhea (AAD). Strains from numerous bacterial species have been tested in clinical studies for mitigating AAD including members of the *Bacillus, Bifidobacterium, Clostridium, Lactobacillus, Lactococcus, Leuconostoc,* and *Streptococcus* genera. Among the fungi, *Saccharomyces boulardii* has also been examined. *Lactobacillus rhamnosus* strain GG and *S. boulardii* strain CNCM I-745 have been most frequently studied [3,4]. A recent (2019) Cochrane review of probiotics for the prevention of pediatric AAD found 33 randomized clinical trials with 6352 participants meeting the inclusion criteria [5••]. This review reported that probiotics conferred a moderate beneficial effect for AAD prevention (number needed to treat for an additional beneficial outcome (NNTB) 9, 95% CI 7 to 13). Consistent with a prior Cochrane review (2015), the risk ratio of developing AAD was significantly reduced when ≥ 5 billion colony forming units (CFUs)/day were consumed [6]. It was suggested that 5 to 40 billion CFU/day of *L. rhamnosus* or *S. boulardii*, the two most commonly applied species, were the most appropriate for preventing AAD in children receiving antibiotics [6]. Nonetheless, the certainty of evidence in the Cochrane review was ranked as moderate because of minor issues with the risk of bias and inconsistency between probiotic strains used [6]. New large, well-designed, multi-centered, randomized trials were recommended [6]. Such studies will be difficult to design because many questions remain on which strains are most effective and on the appropriate timing and duration of use. Understanding the underlying molecular mechanisms of probiotic effects in the gastrointestinal (GI) tract would help to address those questions. Therefore, this review examines what is presently known about the mechanistic basis for probiotic prevention of AAD.

Modulation of gut microbiota composition

Antibiotics cause significant disruptions to the normal composition and functional attributes of the gut microbiome [7]. Such deficits can persist well after the cessation of antibiotic administration [7] and are associated with the development of obesity, asthma, and inflammatory bowel disease (IBD) [8]. Among the numerous impacts of antibiotics on the gut microbiome are reductions in microbial taxonomic richness, diversity, and evenness in the GI tract [7]. Those drastic changes result in a depletion of the normal gut bacterial

residents, and opportunities for colonization by pathogens such as *Clostridium difficile*. Presently, *C. difficile* is predicted to account for about 20% of all AAD cases [9]. However, other opportunistic pathogens, such as *Clostridium perfringens*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and Candida species have also been associated with AAD [10].

Probiotics are assumed to benefit human health by their direct actions on the composition and function of the human gut microbiota [11,12]. However, very few studies on antibiotic use have addressed this possibility (Table 1). In a four-week trial with patients treated for *C. difficile* infection, administration of a four-strain capsule of *Lactobacillus* and *Bifidobacterium* together with antibiotics was associated with significant reductions in the duration of *C. difficile* diarrhea [13]. Examination of fecal contents showed that subjects consuming the probiotic capsules contained lower proportions of *Verrucomicrobiaceae* in their stools compared to those given placebo (empty) capsules [14•] (Table 1). Although few other probiotic-induced differences in bacterial fecal composition were found [14•], the reduced levels of *Verrucomicrobiaceae* were consistent with the positive association that this family has with susceptibility to *C. difficile* infection [15]. In another study initiated after completion of antibiotic treatment for *Helicobacter pylori* infection, there were fewer antibiotic-induced changes to fecal bacterial and fungal composition among subjects taking a multi-strain mixture of *Bacillus subtilis* and *Enterococcus faecium* compared to subjects on a placebo product [16] (Table 1). A similar finding was reported in another study on *H. pylori* treatment, but in that case, the putative probiotic strains were taken during antibiotic use [17].

When antibiotics were administered to healthy volunteers, ingestion of *S. boulardii* CNCM I-745 together with a seven-day regime of amoxicillin-clavulanate was associated with attenuation of microbiota shifts, including less *Escherichia coli* overgrowth [18]. A very different outcome of putative probiotic-mediated effects on the gut microbiome was found in another study wherein healthy volunteers were given a course of ciprofloxacin and metronidazole for 7 days and then administered a 28-day course of an 11-strain mixture or placebo [19]. Consumption of the microbial preparation resulted in potentially negative and persistent consequences on the mucosal and fecal gut microbiome composition (i.e. delayed post-antibiotic reconstitution of the indigenous mucosal microbiome composition and the host GI transcriptome) and a similar effect was observed in mice [19]. However, it should be noted that no clinical endpoints were measured to establish whether that particular study design or strain preparation had measurable consequences on human health.

Animal models have also been used to investigate the extent to which the administration of (putatively) probiotic microorganisms alter antibiotic-mediated changes to the gut microbiome (Table 1). The fecal contents of rats given *Lactobacillus paracasei* CNCM I-3689 prior to, during, and after subcutaneous challenges with clindamycin and oral vancomycin-resistant *Enterococcus faecalis* contained significantly lower levels of vancomycin-resistant enterococci (VRE) and showed a better recovery of members of the phylum *Bacteroidetes* after antibiotic treatment was ended [20]. Other studies focused specifically on administration of the (putative) probiotic microorganisms after antibiotic use [21–23,24•,25•]. Most consistent among the findings was a reduction in the proportions of

Proteobacteria [21–23, 24•, 25•,] and an increase in *Bacteroides* when strains of *Lactobacillus* were consumed [23,24•]. Although several reports noted that *Lactobacillus* consumption did not result in significant changes to the diversity of bacteria in the distal GI tract [21,25•], other studies described an improved recovery of the gut microbiota towards pre-treatment composition [23,24•,26].

The numerous ways that probiotics could modulate gut microbiome composition have been reviewed elsewhere [11]. Specific molecular mechanisms include inhibition of intestinal pathogens by the production of anti-bacterial compounds, competitive exclusion either by the consumption of limited nutrient resources or adherence to the epithelium, or stimulation of indigenous microbial activity (Figure 1). Recently, probiotic *E. coli* Nissle 1917 was found to produce a class of bacteriocins that limit the expansion of competing *Enterobacteriaceae* during intestinal inflammation [27]. The importance of bacteriocins for ecological fitness of bacteria in the GI tract was also shown for Gram-positive bacteria such as *E. faecalis* [27]. Pathogen outgrowth could also be inhibited by competitive exclusion by probiotics for intestinal binding sites. This capacity was indicated by the inhibition of *Campylobacter jejuni* infection by a *Lactobacillus gasseri* SBT2055 (LG2055) cell surface-associated, aggregation-promoting factor which binds to extracellular matrix proteins on intestinal cells [28]. Alternatively, end-products of probiotic metabolism could be consumed by members of the gut microbiota in cross-feeding interactions. For example, a propionogenic bacterial consortium was recently shown to restore fecal propionate levels and alter bacterial composition after antibiotic treatment in a model of the human intestinal microbial ecosystem (M-SHIME) [29•].

Alter nutrient metabolism in the intestine

Antibiotic-mediated, gut microbiome remodeling also results in significant alterations to intestinal metabolomes [30]. Perhaps most important among lumenal metabolite changes is the reduction in short-chain fatty acid (SCFA) levels [31,32]. SCFAs butyrate, propionate, and acetate are the primary end-products of bacterial carbohydrate metabolism in the GI tract and constitute approximately 10% of the human daily caloric requirement [33]. Reductions in SCFA biosynthesis might lead to AAD because these compounds promote NaCl and water absorption [31]. SCFA are rapidly absorbed by the colon and stimulate Na-dependent fluid absorption via a cyclic AMP-independent process with Na-H, SCFA-HCO_3, and Cl-SCFA exchanges [31].

Evidence of probiotic-mediated effects on intestinal SCFA was provided in a human trial whereby *Lactobacillus plantarum* 299V prevented a decrease in SCFA during metronidazole use [26] (Table 1). In mice, *L. rhamnosus* GG was as effective as the butyrate derivative tributyrin at preventing antibiotic-induced intestinal injury and reductions in SCFA receptor (GPR109a) and transporter (SLC5A8) levels [34]. Because lactobacilli lack the pathways necessary for butyrate production, the effect of *L. rhamnosus* GG was likely the result of probiotic-induced, cross-feeding with the gut microbiota to result in increased lumenal butyrate levels or via a butyrate-independent mechanism. In another study, *Lactobacillus acidophilus* ATCC4537-secreted compounds were able to prevent enteropathogenic *E. coli* inhibition of butyrate uptake by Caco-2 cells due to a mechanism that involved prevention of

monocarboxylate transporter isoform 1 (MCT1) endocytosis [35]. Notably, this activity was not found for heat-killed *L. acidophilus* or for three other *Lactobacillus* strains tested [35].

Probiotics could contribute directly to intestinal SCFA by the production of organic acids such as lactate and acetate or by providing a more hospitable environment for SCFA-producing bacteria (Figure 1). Acetate production by probiotic *Bifidobacterium* in the GI tract was shown to reduce the risk for enteropathogenic *E. coli* infection [36]. Probiotic metabolism and production of organic acids (e.g. lactate and acetate) *in situ* could lower lumenal pH and oxygen levels as well as provide substrates used for butyrate and propionate synthesis by other bacterial GI inhabitants [37]. Additionally, growth of probiotic bacteria in the intestine could lead to lower concentrations of undigested carbohydrates, thereby reducing the risk of diarrhea caused by disruptions in osmogradients [31].

Direct modulation of solute secretion and absorption

The lack of solute absorption and/or active solute secretion by the intestinal epithelium results in watery diarrhea. Solute levels are controlled by a variety of basolateral and apical channels and transporters which are responsible for Cl^- secretion and the active transport of Na+ across the epithelium with parallel Cl^- or $HCO3^-$ absorption [38,39]. In mice, *B. subtilis* CU1 (CNCM I2745), but not *L. plantarum* CNCM I-4547, reduced the risk of AAD by inducing the expression of higher quantities of the epithelial Na^+/H^+ exchanger 3 protein NHE3, a protein that promotes fluid absorption, and lower levels of cystic fibrosis transmembrane conductance regulator (CFTR), a protein with a major role in Cl^- secretion [40]. In another study, *L. acidophilus* ATCC4357 prevented *Citrobacter rodentium-induced* diarrhea in mice by counteracting the inhibition of NHE3 [41]. The $Cl^-/HCO3^-$ exchanger protein DRA also remained active with *L. acidophilus* administration [41]. Additionally, *Bacteroides fragilis* ZY-312 [25•] and *L. rhamnosus* GG [34] resulted in the increased expression of genes coding for aquaporin water-channel membrane proteins (Table 1).

Because of the importance of lumenal solute concentrations in diarrhea development, probiotic-mediated alterations to intestinal electrolyte transporters could be a potent mechanism for AAD prevention (Figure 1). Besides SCFAs (discussed above), other compounds might confer similar effects as was recently shown for gassericin A, a bacteriocin made by *L. gasseri* and *Lactobacillus frumenti* [42••]. Wild-type *L. gasseri,* but not an isogenic mutant deficient in gassericin A synthesis, was able to prevent diarrhea in piglets. Testing of the purified bacteriocin *in vitro* showed that it increased intestinal fluid absorption as a result of inducing higher cellular cyclic nucleotide levels in epithelial cells via mechanism involving binding to the membrane protein Keratin 19 (KRT19) and activating mTOR (mechanistic Target of Rapamycin) phosphodiesterase activity [42].

Increase secondary bile acid concentrations

In healthy individuals, approximately 95% of lumenal bile acids are reabsorbed in the distal ileum [43]. The remaining amounts are modified by intestinal bacteria and then are either excreted or passively absorbed [43]. Antibiotics disrupt this process and result in increases in colonic primary bile acids, compounds that inhibit epithelial ion transport proteins [43].

Reductions in microbially-modified, secondary bile acids also increase susceptibility to *C. difficile* infection [44].

The potential for probiotics to alter bile acid composition concurrent with antibiotic consumption was shown in healthy volunteers given *S. boulardii* CNCM I-745 [45•] (Table 1). Fecal samples from individuals on amoxicillin-clavulanate contained higher quantities of cholic acid, a primary bile acid, and lower levels of secondary bile acids. Those changes were reversed in subjects taking *S. boulardii* CNCM I-745 [45•]. Although more studies are needed to assess the relative importance of bile acid metabolism on probiotic prevention of AAD, such outcomes might occur either by direct modification of bile acids by probiotic microbes or by broader effects which result in the maintenance/enrichment of certain members of the gut microbiome (Figure 1). Direct modification of bile acids with bile salt hydrolases (BSH) by *Lactobacillus, Bifidobacterium,* and *Clostridium* species is already well known. BSHs deconjugate bile acids and the resulting compounds can then be further modified to secondary and tertiary bile acids by other intestinal bacteria [43]. A functional role for intestinal BSH was demonstrated in mice whereby it was shown that higher BSH activity resulted in systemic responses mitigating cardiometabolic impacts of a high fat diet [46]. Recently, it was determined that *bsh* genes are enriched among vertebrate-associated *Lactobacillus* species [47]. An intestine-associated BSH phylotype with the highest enzymatic activity was only found in *Lactobacillus* and not other members of the human gut microbiome [48•].

Improve intestinal barrier function

Intestinal epithelial barrier integrity is increasingly understood to be important for the pathology of a number of intestinal and systemic diseases [49]. Antibiotics induce deficits in barrier function, or a “leaky gut”, according to studies with rodent models (Table 1) but the severity of the barrier losses appears to vary depending on the antibiotic used [50]. The capacity of certain, administered bacteria to prevent antibiotic-induced disruptions to the intestinal epithelium was demonstrated in animal studies (Table 1). A strain of *Bacillus amyloliquefaciens* was associated with improved structural and functional aspects of small intestine tissues in piglets given aureomycin [51]. Similar findings were reported for *Lactobacillus casei* CGMCC 12435 and a mixture of *Lactobacillus* and *Bifidobacterium* strains given to mice after ampicillin treatment [23,24]. Those results were supported by the observed increases in transcripts for tight junction proteins [23,24,51]. A high dose of *B. fragilis* ZY-312 (daily administration of 10^9 CFU) was associated with colonic increases in ZO-1 and occludin tight junction proteins, mucin synthesis, and cell markers for epithelial cell proliferation [25•]. Currently, specific compounds responsible for probiotic-induced changes to epithelial barrier function are largely unknown [52]. Recently, we have shown that the bacteriocin Plantaricin EF produced by *L. plantarum* can prevent pro-inflammatory cytokine mediated deficits to barrier integrity in *in vitro* and that wildtype *L. plantarum,* but not a plantaricin-deficient mutant strain, increases intestinal ZO-1 synthesis in obese mice [53•]. Other extracellular bacterial proteins such as the outer membrane pilus-associated protein synthesized by *Akkermansia mucinophilia* can also confer improvements to the intestinal barrier [54].

Alter intestinal immune responses

Antibiotics also affect immune homeostasis. In human subjects, antibiotic use resulted in impaired vaccine responses among individuals with low pre-existing antibody titers [55]. Antibiotics were also found to induce long-term, macrophage-dependent increases in inflammatory T helper 1 (TH1) responses in mice and heightened susceptibility to some infections [56].

Putative and established probiotic bacteria and yeast were found to counter antibiotic activation of inflammatory pathways in humans [19], piglets [51], and mice [23,24] (Table 1). Reductions in C-reactive protein, Complement C3, and IgG with administered *Lactobacillus* strains [24] indicate that those microbes might be able to limit the systemic effects of antibiotics (Table 1). These findings are consistent with strain-specific immunomodulation capacities of probiotics in healthy human subjects and individuals with chronic immune-associated diseases (e.g. allergy, asthma) [57,58]. Although the specific probiotic cell products able to directly alter immune cell function during antibiotic use are not yet known, recent reports show that certain extracellular compounds, such as *Bifidobacterium* exopolysaccharides [59] and *Lactobacillus* Slayer proteins [60] are immunomodulatory. Therefore, there are likely multiple secreted compounds made by probiotics which could influence immune system during antibiotic administration (Figure 1).

Conclusions

Results from clinical trials support the use of probiotics for preventing AAD. Therefore, it is notable that very few studies have investigated the molecular basis for probiotic AAD prevention (Table 1). Most reports have focused on strains that are not commercially available and are poorly characterized. Moreover, very few mechanistic studies with humans and animal models have directly examined *L. rhamnosus* GG or *S. boulardii* CNCM I-745, the strains most commonly tested in human trials [6] (Table 1). For these reasons, it is not yet possible to report which gut-modulating activities of probiotic microorganisms are the most important for protecting against AAD. Just as AAD is result of multiple factors connected with antibiotic administration (e.g. disruption of the gut microbiota, decreased intestinal SCFA concentrations, accumulation of luminal carbohydrates and colonic bile acids, altered water absorption), it is most likely that probiotic effects are multi-factorial and are both strain- and host-background dependent. It is also expected that there will be mechanistic overlap between strains (e.g. effects due to the production of organic acids) as well as strain-specific, host-microbe interactions (e.g. effects due to secretion of strain-specific enzymes and proteins) [61]. Such effects could be assessed in well-controlled, multi-center clinical trials for which intestinal and gut microbiota responses are measured and combined with complementary animal model studies using the same protocols. Elucidating the molecular mechanisms of probiotic action in the gut is extremely important for developing recommendations for existing strains such as the recommended dose, frequency, and duration of a probiotic intervention, the value of using multi-strain formulations, and the optimal protocols and ingredients for probiotic manufacture and carrier delivery. This knowledge is also needed for designing the appropriate assays to select the next-generation probiotics.

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•• of outstanding interest

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Highlights:

- Clinical studies support the use of probiotics for preventing AAD.
- AAD prevention by probiotics is multifactorial and strain dependent.
- Probiotics are associated with gut microbiome modulation upon antibiotic use.
- Intestinal bile acids and SCFAs are implicated in probiotic prevention of AAD.
- Probiotic effector compounds may regulate intestinal fluid secretion and absorption.

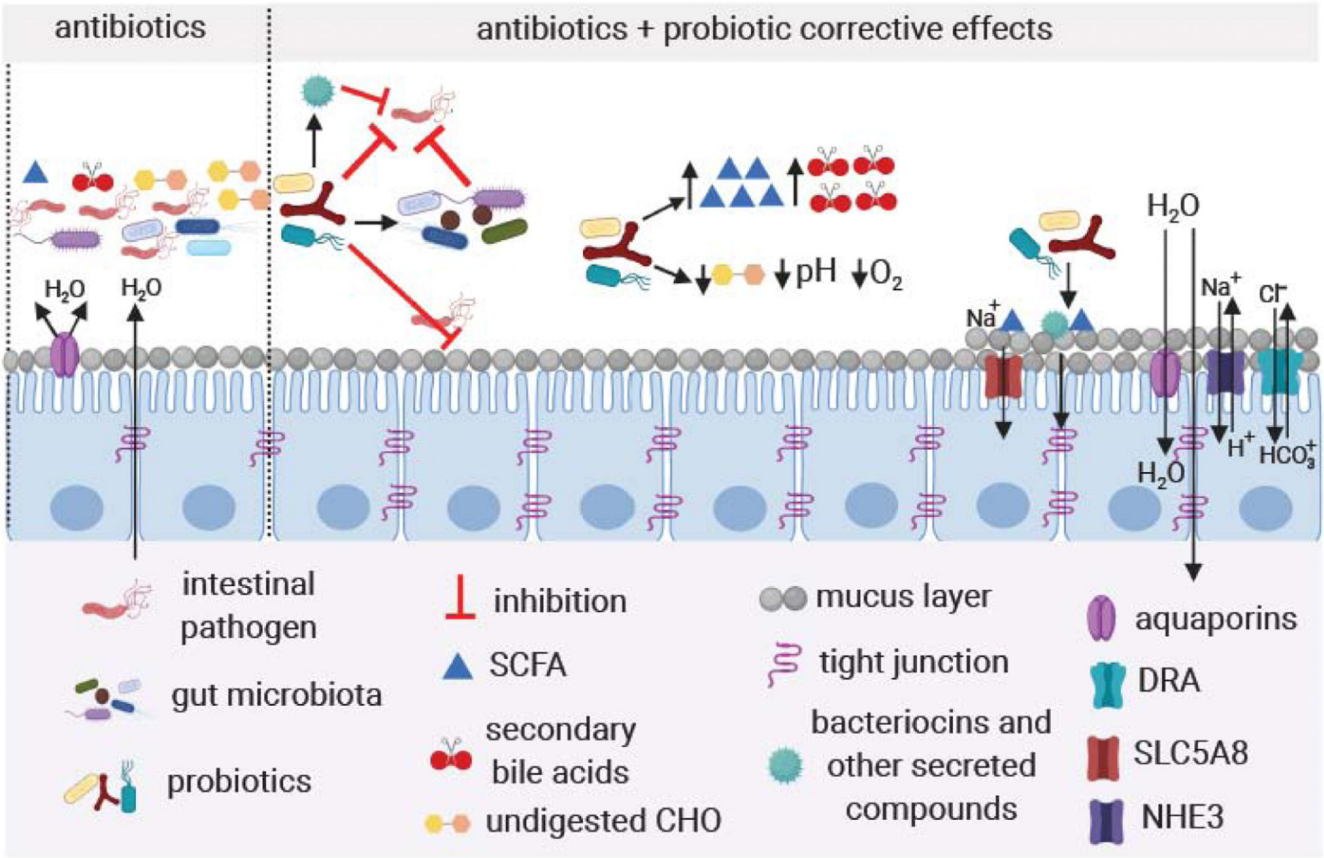

Figure 1. Schematic model of the potential molecular mechanisms responsible for probiotic prevention of AAD.

Antibiotic treatment disrupts the composition of the GI tract microbiota, leading to increased growth of opportunistic pathogens, the accumulation of undigested carbohydrates, and reduced levels of SCFAs and modified bile acids. Probiotics might counter antibiotic-induced effects in the GI tract by directly impairing pathogen growth or by inducing other alterations to gut microbiota composition via SCFA synthesis, production of other secreted metabolites such as bacteriocins, or by reducing lumenal pH and O2 levels. Probiotics might also cause changes to bile acid composition as well as directly interact with the intestinal epithelium and immune system to result in increased gut barrier function and modulation of water and solute transport.

Table 1.

Human and animal antibiotic studies measuring the effects of probiotics on intestinal responses

			Responses to probiotics compared to antibiotic treatment alone			
	Antibiotic	**Strain(s) and study design**	**Gut microbiota and metabolome**	**Intestinal protein and gene expression**	**Physiologica l responses**	
Humans	Vancomycin or metronidazole; *C. Difficile* treatment	*L. acidophilus* ncfm, *L. paracasei* Lpc-37, *B. lactis* Bi-07, and *B. lactis* B1–04; concurrent with antibiotics	No change in bacterial alpha- or beta-diversity ↓ *verrucomicrobiaceae* ↑ *bacteroides*	ND	↓ Duration and total diarrhea No change in *C. difficile* recurrence	[13,14•]
	Clarithromycin, amoxicillin and esomeprazole; *H. pylori* treatment	*B. subtilis* and *E. faecium* (strain designations not provided); post-antibiotic treatment	↓ Changes in bacterial and fungal composition over time	ND	No change in *H. pylori* eradication; ↓ Reduced incidence of side-effects (e.g. diarrhea)	[16]
	Clarithromycin, amoxicillin, lansoprazole for *H. pylori* treatment	*B. subtilis* and *Streptococcus faecium* (strain designations not provided); concurrent with antibiotics	↑ Bacterial alpha-diversity ↓ Changes in bacterial composition compared to baseline ↑ Genis for amino acid and sugar metabolism ↓ Genes for seleno-compound metabolism	ND	ND, small sample size	[17]
	Amoxicillin-clavulanate; Healthy volunteers	*S. boulardii* CNCM I-745' conc. Tern with antibiotics	↓ *Escherichia* ↓ Parabacteroides and Ralstonia ↑ Fecal secondary bile acids	ND	↓AAD scores	[18,45•]
	Ciprofloxacin and metronidazole; Healthy volunteers	11-strain mixture ith species of *Lactobacillus*, *Bifidobacterium, Lactococcus*, and *Streptococcus* [a]; post-antibiotic treatment	↓ Recovery of the indigenous microbiome (fecal and mucosal), including: ↓ Bacterial alpha-diversity; ↓ Bacterial load; ↓ *Clostridiales*	↑ REG3G, IL1B transcripts	ND	[19]
	Metronidazole; *C. difficile* treatment	*L. plantarum* 299V; concurrent with antibiotics	Non-significant reductions in SCFA	ND	Slight reduction in recurrence of clinical symptoms (small sample size)	[26]
Piglets	Aureomycin	*Bacillus amyloliquefaciens* SC06; concurrent with antibiotics	ND	↑ IL-6, MyD88, NOD-1, TLR4, and ↓ TNFα transcripts in jejunum ↑ TNFα protein in serum ↑ TNFα, IFN-γ, IL-6, and IL-10 in liver	↑ Intestinal villus height ↓ crypt depth ↓ Gt permeability	[51]
Rats	Clindamycin, ampicillin. streptomycin	*B. fragilis* ZY-312 in different doses (10^7, 10^8, 10^9 CFU/day) and/or a mixture of	No change in bacterial alpha-diversity ↑ *Akkermansia* ↓ *Escherichia*	↑ Colon *Aqpi, Aqp3* and *Aqp8* transcripts	↓ Fecal water and ↑ fecal consistency score with the	[25•]

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	Antibiotic	Strain(s) and study design	Responses to probiotics compared to antibiotic treatment alone: Gut microbiota and metabolome	Intestinal protein and gene expression	Physiologica l responses	
		Bifidobacterium longum, L. acidophilus, and *E. faecalis* post-antibiotic treatment		↑ Mucin 2 transcripts, colon ZO-1, occludin and ↑ Ki-67 positive cells with the highest dose of *B. fragilis* ZY-312	highest dose of *B. fragilis* ZY-312	
Mice	Clindamycin (subcutaneous) and *E. faecalis* V583	*L. paracasei* CNCM I-3689; concurrent with antibiotics	↓ Vancomycin-resistant enterococci ↑ *Bacteroidetes*	↑ Ki67 and PCNA-positive cells ↑ *camp* (cathelicidin LL-37) transcripts	ND	[20]
	Metronidazole, neomycin sulfate and vancomycin	*L. rhamnosus* GG; concurrent with antibiotics	ND	↑ GPR109a, SLC5A8, SLC26A3, AQP4, and NHE3 transcripts	No effect on mouse weight	[34]
	Cefixime	Two strain mixtures with *L. plantarum, L. casei, L. rhamnosus*, and *Lactobacillus helveticus* [b]; post-antibiotic treatment	↑ Recovery of microbiota composition: ↑ *Firmicutes* ↓ *Bacteroidetes* ↑ *Proteobacteria* ↑ SCFA	↓ CRP, C3, and IgG	↓ Cecum size ↓ Inflammatory infiltrate	[22]
	Ampicillin, streptomycin, clindamycin	*L. rhamnosus A191, L. acidophilus, Bifidobacterium breve* and *B. longum* (no designations); post-antibiotic treatment	No change in bacterial alpha-diversity ↓ *Enterobacteriaceae*, ↑ *Firmicutes*	ND	ND	[21]
	Ampicillin	Separate groups given *L. casei* CGMCC 12435 (LacC), *L. plantarum* CGMCC 12436 (LacP), or *L. rhamnosus* GG (LacG); post-antibiotic treatment	↑ Recovery of microbiota composition: ↑ alpha-diversity ↑ *Bacteroidetes* ↓ *Proteobacteria* (LacC and LP) ↑ SCFA (LacC)	↑ Ileal ZO-1, occludin (LacC), Claudin- 1(LacP) transcripts ↓ NF-κB (LacC, LacP) ↓ IL-1β, IFN-γ ↓ (LacC) ↓ Reg-3γ ↓ (LacG), ↓ sIgA (LacC, LacG)	↓ Gut permeability (LacC) ↓ Serum endotoxin and diamine oxidase	[24•]
	Ampicillin	Mixture of *L. casei* CCFM2710, *L. plantarum* CCFM2602, *L. rhamnosus* CCFM492, and *L. helveticus* CCFM671; post-antibiotic treatmen.	↑ Recovery of microbiota composition: ↓ *Firmicutes* and ↑ *Bacteroidetes* ↓ *Proteobacteria* in stools	↑ Ileum and colon ZO-1, occludin and claudin-1 transcripts ↓ Ileum TNF-α, IL-6, MCP-1and IFN-γ	↓ Gut permeability ↓Serum endotoxin and D-lactate levels	[23]

ND: Not Determined

Abbreviations: C3: Complement 3, sIgA: secretory immunoglobulin A, aqp: aquaporin, GPR109A: G-protein-coupled receptor 109A, SLC5A8: Solute Carrier Family 5 Member 8, SLC26A3: Solute Carrier Family 26 Member 3, and NHE3: Na+/H+ exchanger 3

[a] For reference [19], a multistrain mixture was used as follows: *L. acidophilus* ATCC4356, *L. rhamnosus* (strain designation not provided), *L. casei* ATCC393, *L. paracasei* ATCC BAA-52, *L. plantarum* ATCC8014, *Bifidobacterium longum* subsp *infantis* ATCC15697, *Bifidobacterium bifidum* ATCC29521, *Bifidobacterium breve* ATCC15700, *Bifidobacterium longum* subsp. *longum* ATCC15707, *Lactococcus lactis* (strain designation not provided), *Streptococcus thermophilus* ATCC BAA-491

[b] For reference [22], a multistrain mixture was used as follows: L. plantarum including CCFM4, CCFM10, CCFM595, CCFM602, and CCFM605; L. casei CCFM5, CCFM30, CCFM236, CCFM2710 and CCFM2711; L. rhamnosus LGG, CCFM237, CCFM311, CCFM319 and CCFM492; and L. helveticus CCFM6, CCFM672 and CCFM673.