Gut Dysbiosis and Clostridioides difficile Infection in Neonates and Adults

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In this review, we focus on gut microbiota profiles in infants and adults colonized (CDC) or infected (CDI) with Clostridioides difficile. After a short update on CDI epidemiology and pathology, we present the gut dysbiosis profiles associated with CDI in adults and infants, as well as the role of dysbiosis in C. difficile spores germination and multiplication. Both molecular and culturomic studies agree on a significant decrease of gut microbiota diversity and resilience in CDI, depletion of Firmicutes, Bacteroidetes, and Actinobacteria phyla and a high abundance of Proteobacteria, associated with low butyrogenic and high lactic acid-bacteria levels. In symptomatic cases, microbiota deviations are associated with high levels of inflammatory markers, such as calprotectin. In infants, colonization with Bifidobacteria that trigger a local anti-inflammatory response and abundance of Ruminococcus phyla and a high abundance of Proteobacteria, associated with low butyrogenic and high lactic acid-bacteria levels. In symptomatic cases, microbiota deviations are associated with high levels of inflammatory markers, such as calprotectin. Gut dysbiosis amelioration through administration of “biotics” or non-toxigenic C. difficile preparations and fecal microbiota transplantation proved to be very useful for the management of CDI.

Keywords: Clostridium difficile infection, gut microbiota, dysbiosis, biotics, fecal microbiota transplantation

INTRODUCTION

Clostridioides (formerly Clostridium) difficile is a Gram-positive, obligate anaerobe, spore-forming bacteria, harboring a plethora of surface and secreted proteins responsible for the colonic colonization and subsequent inflammation characteristic for C. difficile infection (CDI), among which the most important are the clostridial toxins: toxin A (TcdA) and toxin B (TcdB), and in some bacterial strains, the binary toxin CDT (Smits et al., 2016). Clinical symptoms range from mild diarrhea to fulminant colitis, known as pseudomembranous colitis, with its complications – toxic megacolon and large bowel perforation (McDonald et al., 2018; Dieterle et al., 2019). C. difficile is the number one causative agent of nosocomial post-antibiotic colitis, associated with high
morbidity and mortality (Ghose, 2013). In the last decade, both frequency and severity of CDI have increased, largely due to the emergence of a hypervirulent strain called NAP1 (North American pulsed-field gel electrophoresis type 1 strain) (Depestel and Aronoff, 2013). Moreover, in the last two decades, there has been a significant increase in the incidence of CDI in previously considered low-risk population categories, including community-associated-Clostridioides difficile infections (CA-CDI), with more than 30% of cases not showing typical CDI risk factors, such as antibiotic treatment or recent hospitalization (Wilcox et al., 2008; Hensgens et al., 2011). Although CDI is the preserve of the elderly population, it can also affect other age segments. In a retrospective survey performed by U.S. National Hospital Discharge Surveys from 2001 to 2010 revealed that CDI incidence was highest among elderly adults (11.6 CDI discharges/1,000 total discharges), followed by adults (3.5 CDI discharges/1,000 total discharges) and pediatrics (<12 years) (1.2 CDI discharges/1,000 total discharges). The mortality rates attributable to CDI in the elderly were significantly higher (8.8%) compared to adults (3.1%) and pediatrics (1.4%) (Pechal et al., 2016). Although there is not much information available about the epidemiology of infection in infants, however, the carriage rate of non-toxigenic C. difficile is very high in newborns, suggesting the commensal status of this bacterium in this population segment (Sammons et al., 2013; Borali and De Giacomo, 2016). For this reason, C. difficile is not considered an enteric pathogen in infants and children affected by bloody diarrhea and younger than 12 months (Cama et al., 2019). However, asymptomatic infants could be also infected by toxigenic adult infectious strains both after hospitalization and even in the community, thus, constituting a reservoir for toxigenic strains (Rousseau et al., 2011; Ferraris et al., 2020).

This review aims to present some particular aspects of gut microbiota in CDI infants and adults, taking into account that the pathophysiology of this disease suggests that the clinical manifestations occur in cases of an imbalance of the intestinal microbiota, known as dysbiosis. In this purpose, studies performing culture-dependent (C. difficile cultivation) and independent (16S rRNA and metagenomics) have been analyzed.

Some of the underlying causes of intestinal dysbiosis are antibiotic treatments (Kukla et al., 2020), advanced age (over 65 years), hospitalization (particularly in patients sharing the hospital room with an infected patient, in intensive care units, during prolonged hospitalization), nursing home stay, severe associated diseases, immunological suppression, gastric acidity suppression by proton pump inhibitors or histamine-2-receptor antagonists and prolonged use of elemental diet in the context of enteral nutrition, inflammatory bowel diseases, gastrointestinal surgery (in particular colectomy, small-bowel resection, and gastric resection were associated with the highest risk while patients undergoing cholecystectomy and appendectomy had the lowest risk), all of these circumstances being associated with characteristic changes in the configuration of the gut microbiota and with an increased CDI risk (O’Keefe, 2010; Fashner et al., 2011; Nitzan et al., 2013; Rodriguez et al., 2016; Sartelli et al., 2019; Avni et al., 2020). A strong argument regarding the impact of dysbiosis on CDI risk is offered by animal studies proving that correcting dysbiosis by administration of different substances such as phytophenolic compounds or carvacrol has been shown to decrease susceptibility to CDI. The gut dysbiosis of 6-week-old C57BL/6 mice was infected with the oral administration of an antibiotic cocktail in water simultaneously with the intra-peritoneal injection of clindamycin. The mice were infected with $10^5$ CFU/ml of hypervirulent C. difficile ATCC 1870 spores. Carvacrol supplementation significantly reduced the incidence of diarrhea and improved mice’s clinical and diarrhea scores. Microbiome analysis revealed that carvacrol increased the abundance of Bacteroidetes and Firmicutes. An increased abundance of Lactobacillaceae and Lachnospiraceae was noticed among the beneficial taxa in carvacrol treated mice. Also, carvacrol decreased the proportion of pro-inflammatory microbiota, such as Proteobacteria (i.e., Enterobacteriaceae) and Verrucomicrobia, without significantly affecting the gut microbiome diversity compared to the control (Mooyottu et al., 2017).

Five clinical features (potential risk factors) predict dysbiosis in CDI patients: antibiotic use within the previous 3 weeks, immunosuppression, multimorbidity, recent/multiple hospitalization, and prior CDI (Battaglioli et al., 2018). In individuals whose normal intestinal microbiota has been disrupted, ingested C. difficile spores germinate in the presence of bile salts in the small intestine and colonize the colon epithelial cells, releasing the inflammatory enterotoxins, which are primarily and largely responsible for the colonic inflammation in C. difficile diseases, inducing cytoskeletal changes, disruption of tight junctions, and induction of inflammatory cytokine production (Shen, 2012; Winston and Theriot, 2016). The C. difficile spores are released by the patient facilitating CDI transmission to susceptible hosts (Czepiel et al., 2019).

Clostridioides difficile INFECTION AND DYBSIOSIS IN ADULTS

The intestinal environment represents a complex network of bacterial cells and metabolic products and/or other unknown substances derived from their own structures or metabolisms, which are in close and continuous interaction, both with each other, as well as with the human intestinal cells and the host’s immune system (Lazar et al., 2018, 2019).

The characterisation of the baseline healthy microbiota and differences that are associated with various diseases has been possible with the contribution of large-scale projects, such as Meta-HIT and the Human Microbiome Project (HMP), using different omics technologies (Qin et al., 2010; Human Microbiome Project, 2012).

During early development, the gut microbiota undergoes subsequent changes until a stable adult state is reached. The adult microbiota has three basic characteristics: diversity (a high microbiota diversity defined by high species richness and high functional diversity being generally associated with the health condition), resilience (the property of the gut microbiota to resist to an impact and to recover and to baseline after the disturbance cessation; the capacity of a microbial community to reach a stable
TABLE 1 | Gut microbiota dysbiosis associated with *Clostridium difficile* infection in adults and infants (proposed microbiota-derived biomarkers for CDI dysbiosis are presented in bold).

| Effect | Taxonomic level | Representatives | Mechanism | References |
|-------|----------------|----------------|-----------|------------|
| Adults | Depletion Gut microbiota | Cultivable/non-cultivable microbiota | Disrupted microbiota; decreased richness and diversity | Amrane et al., 2019 |
|       | Phylum Firmicutes | Butyrate and short chain fatty acid production; role in gut homeostasis and inhibition of *C. difficile* germination | Antharam et al., 2013; den Besten et al., 2013; Abt et al., 2016 |
|       | Bacteroidetes | Carbohydrate digestion, producing substrates for colonocytes | den Besten et al., 2013; Zhang et al., 2015 |
|       | Actinobacteria | Colonization resistance | Amrane et al., 2019; Lozupone et al., 2012 |
|       | Families Bacteroidaceae | | Reeves et al., 2011, 2012; Antharam et al., 2013; Perez-Cobas et al., 2015 |
|       | Bifidobacteriaceae | | |
|       | Lachnospiraceae | | |
|       | Clostridiales Faecalibacterium, Roseburia, Blautia, Dorea, Prevotella, Megamonas, Subdoligranulum, Anaerostipes, Pseudobutyrylbacter, Streptococcus, Enskelliella, Odoribacter, Bacteroides sp., Alistipes, *B. ovatus*, *B. vulgatus*, *Bifidobacterium adolescentis*, *B. longum*, Oscillibacter massiliensis, Clostridium scindens | Decrease of luminal pH by butyrogenic and acetogenic bacteria, stimulation of mucin and antimicrobial peptides production, maintaining decreased permeability Primary bile acids conversion Production of lantibiotics (nisin O) | Guilloteau et al., 2010; Lozupone et al., 2012; Antharam et al., 2013; Hamilton et al., 2013; Solomon, 2013; Gupta et al., 2016; Milani et al., 2016; Theriot et al., 2016; Winston and Theriot, 2016; Hatzieanou et al., 2017; Vakili et al., 2020 |
|       | Ruminococcaceae | | |
|       | Genera and species Faecalibacterium, Roseburia, Blautia, Dorea, Prevotella, Megamonas, Subdoligranulum, Anaerostipes, Pseudobutyrylbacter, Streptococcus, Enskelliella, Odoribacter, Bacteroides sp., Alistipes, *B. ovatus*, *B. vulgatus*, *Bifidobacterium adolescentis*, *B. longum*, Oscillibacter massiliensis, Clostridium scindens | Decrease of luminal pH by butyrogenic and acetogenic bacteria, stimulation of mucin and antimicrobial peptides production, maintaining decreased permeability Primary bile acids conversion Production of lantibiotics (nisin O) | |
| Infants | Increase Phylum Proteobacteria | | Rodriguez et al., 2020 |
|         | Families Enterobacteriaceae | Increased intestinal permeability | Collins and Auchtung, 2017 |
|         | Genera/species Finegoldia, Enterococcus, Lactobacillus, Fusobacterium, Mycobacterium, Enterobacter, Bacteroides, Parabacteroides, Escherichia coli, Akkermansia muciniphila | Lactic acid bacteria | Reeves et al., 2011, 2012; Na and Kelly, 2011; Rea et al., 2012; Rajilic-Stojanovic et al., 2013; Gevers et al., 2014; Milani et al., 2016; Ross et al., 2016 |
| Infants | Increase Genera/species Staphylococcus aureus, Enterococcus, Escherichia coli, Shigella spp., Citrobacter spp., Klebsiella spp. | Triggering a pro-inflammatory response | Heida et al., 2016 |
| Infants | Decrease Phylum Bacteroidetes, Firmicutes | Uregulation of IL-10 production | Dobbler et al., 2017 |
| Infants | Genera/species Ruminococcus | | Huurre et al., 2008 |

state in response to chemical, physical or biological perturbations of different intensities is achieved through genetically diverse resident clonal populations and population-level dynamics) and long-term stability of high taxonomic level components (Levine and d’Antonio, 1999; Lozupone et al., 2012; McBurney et al., 2019; Priya and Blekhman, 2019; Dogra et al., 2020).

Regarding the diversity, human microbiota displays a remarkable heterogeneity within and between individuals, the results of the culture-independent studies leading to the generally accepted idea that we rather share a functional core microbiome, than a core microbiota (Lozupone et al., 2012). The >1,000 estimated species-level phylotypes are belonging to few microbial phyla, which are *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*; among these, the two *Firmicutes* and *Bacteroidetes* phyla are representing 90% of gut microbiota (Magne et al., 2020). *Firmicutes* comprises more than 200 different genera, such as *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminococcus* (Kachrimanidou and Tsintarakis, 2020). *Clostridium* genera represent 95% of the *Firmicutes*, while *Bacteroidetes* include as predominant genera *Bacteroides* and *Prevotella* (Rinninella et al., 2019). *Actinobacteria* are proportionally less abundant and mainly...
represented by Bifidobacterium species (Rinninella et al., 2019). From the mentioned components, Ruminococcus and Bifidobacterium have been reported to exhibit protective roles against CDI (Seekatz and Young, 2014; Kho and Lal, 2018).

The gut microbiota community shows resilience to different perturbations, such as those induced by different diets, antibiotic administration, invasion by new species (called colonization resistance) (Folke et al., 2004). Under the impact of a certain disturbances, such as antibiotic administration, microbiota enters an unstable state that progresses to a new stable state. When the latter is highly similar to the pre-disturbance state, this indicates a complete recovery. However, sometimes the post-disturbance stable state is distinct and this unfortunately can be both abnormal and resilient, as a response to the perturbation persistence (e.g., poor diet, antibiotic treatments etc) (Dupont, 2011). An example of gut microbiota resilience is the success of bacteriotherapy or microbiota transplantation in treating recurrent CDI. In this case, the gut microbiota switches from an initial dysbiosis state, favoring the CDI (e.g., increased abundance of Veillonella and Streptococcus) to a baseline state, in which the taxa from the healthy donor (e.g., Bacteroidetes) persisted 1 month after transplantation (Gough et al., 2011).

A high diversity (high species richness, α-diversity) and host immune effectors, e.g., nucleotide-binding oligomerization domain (Nod2), an intracellular innate immune sensor involved in the anti-infectious host defense, were linked to gut microbiota resilience (Tap et al., 2015; Raymond et al., 2016; Goethel et al., 2019). A low microbiota diversity was correlated with recurrent CDI, but with unknown effects on microbiota resilience (Chang et al., 2008; Khoruts et al., 2010). However, a comparable phylum-level diversity was observed in individuals with initial CDI and healthy controls, while in case of recurrent CDI the phylum-level diversity switched to different highly divergent profiles very different from the healthy state other (Chang et al., 2008).

The stability of gut microbiota is affected by different factors, including genetic factors, early-life events, travel, dietary changes, weight loss or gain, diarrheal disease, antibiotics, immunosuppressants, premeditated interventions to influence the microbiota by administration of prebiotics, probiotics, postbiotics and symbiotics, as well as fecal transplantation (Faith et al., 2013; Britton and Young, 2014).

Dysbiosis Profiles in Clostridioides difficile Infection Patients

In this section, we will present some of the culture-dependent and independent studies that have been performed to identify the dysbiosis profiles (Table 1) and specific microbial derived biomarkers in patients prone to CDI. A metagenomic and culturomic analysis of gut microbiota dysbiosis during CDI has shown a significant depletion of Bacteroidetes in C. difficile patients compared with the control group (Amrane et al., 2019). Diversity was significantly higher in the control group. Proteobacteria were more common in the CDI group. Firmicutes and Actinobacteria were less common in the CDI group (Lozupone et al., 2012). Firmicutes are involved in butyrate, and other short chain fatty acids (SCFA) production, these molecules playing a role in gut homeostasis and inhibition of C. difficile germination (Kachrimanidou and Tsintarakis, 2020) and Bacteroidetes are involved in carbohydrates digestion, producing substrates for colonocytes (den Besten et al., 2013). Depletion of these two major phyla of gut microbiota was detected in the C. difficile group. The bacterial families conferring resistance to CDI are Bacteroidaceae, Bifidobacteriaceae, and Lachnospiraceae (Antharam et al., 2013). Studies in animals with CDI, revealed a high abundance of Proteobacteria (especially Enterobacteriaceae) and a numerical decrease of Lachnospiraceae (Firmicutes) in diseased animals (Reeves et al., 2011). Lachnospiraceae strains have been shown to be able to partially restore colonization resistance, the mice inoculated with such strains showing decreased C. difficile colonization, lower levels of cytotoxins and lower clinical signs of severe infection (Reeves et al., 2012).

An increased relative abundance of Enterococcus, Lactobacillus, Escherichia coli, Enterobacter, Bacteroides, Parabacteroides, Akkermansia muciniphila, and decreased Faecalibacterium, Roseburia, Blautia, Prevotella, Megamonas, Streptococcus, and Bacteroides levels were evidenced in the gut microbiota of CDI patients (Rea et al., 2012). The bacteria found only in the control group, which may have a role against C. difficile, were Bacteroides ovatus, Bacteroides vulgatus and Oscillibacter massiliensis (Lozupone et al., 2012). Only three bacteria with a potential role against C. difficile were detected both by culturomics and metagenomics, namely Bifidobacterium adolescentis, Bifidobacterium longum and Bacteroides ovatus (Lozupone et al., 2012). Overrepresentation of Akkermansia may be a predictive marker for the development of nosocomial diarrhea, with a worsened CDI prognosis (Hernandez et al., 2018; Vakili et al., 2020).

Dysbiosis-Associated Biochemical Features in Clostridioides difficile Infection Patients

The gut dysbiosis also results in biochemical and immunological disruptions like decreased short chain fatty acids (SCFAs) levels, the abundance of primary bile acids, high availability of carbohydrates, suppression of immunological mechanisms and absence of competitors, all resulting in increased colonization capacity, favoring germination and growth of C. difficile (Ridlon et al., 2014; Carding et al., 2015; Goh and Klaenhammer, 2015; Valdes et al., 2018; Hills et al., 2019; Toor et al., 2019). Nosocomial diarrheal syndromes, including CDI, were associated not only with decreased bacterial diversity, Firmicutes paucity, low numbers of Ruminococaceae, Lachnospiraceae but also with low levels of butyrogenic (e.g., Roseburia, Faecalibacterium, Subdoligranulum, Anaerostipes, and Pseudobutyrivibrio) and acetogenic (e.g., Blautia and Dorea) genera and with high levels of lactic acid bacteria (e.g., Enterococcus sp.) (Antharam et al., 2013). A decrease in butyrate-producing bacteria and an increase in lactic acid-producing bacteria were associated with increased CDI risk (Vakili et al., 2020). The role of SCFAs depletion in facilitating C. difficile infection is not yet elucidated, yet however, SCFAs could act by reducing the luminal pH (unfavorable for C. difficile) and stimulating the defensive barrier.
by production of mucin and antimicrobial peptides (defensins and cathelicidins, secreted by specialized cells, Paneth cells and leukocytes in the intestinal crypts) (Guilloteau et al., 2010; Solomon, 2013; Gupta et al., 2016). Among SCFAs, butyrate seems to have no effect on C. difficile colonization and toxin production, but it can protect the intestinal epithelium from the damage caused by C. difficile toxins by stabilizing hypoxia-inducible factor-1 (HIF-1) and increasing tight junctions, and thus decreasing intestinal epithelial permeability, inhibiting intestinal inflammation and bacterial translocation. The addition of butyrate to the drinking water of mice, administration of a pro-drug of butyrate, tributyrin, or of an inulin-rich diet (inulin can be fermented by gut commensal bacteria, which generate short-chain fatty acids, mainly acetate, propionate, and butyrate) resulted in the protection of mice against CDI (Fachi et al., 2019; Song et al., 2020).

C. difficile spore germination is regulated by the detection of bile salt and amino acid cogerminants by pseudoproteases CspC and CspA, respectively (Weingarden et al., 2014; Lawler et al., 2020). Although some cholate derivatives and the amino acid glycine could act as co-germinant factors, deoxycholate prevents vegetative growth (Sorg and Sonenshein, 2008), while chenodeoxycholate inhibits taurocholate-mediated germination (Sorg and Sonenshein, 2010). Commensal members of Clostridiales present in the gut contribute to the creation of an inappropriate environment for C. difficile germination and colonization by modulating the production of cogerminants (Perez-Cobas et al., 2015). For example, Clostridium scindens, a bile acid 7α-dehydroxylation intestinal strain, is associated with resistance to C. difficile infection, and, upon administration, it enhances resistance to infection in association with a secondary bile acid (Buffie et al., 2015). Depleting specific gut microbes responsible for converting primary bile acids into secondary antimicrobial bile acids could be associated with increased risk of CDI (Theriot et al., 2016; Winston and Theriot, 2016).

Clostridium species are among the best-described users of free amino acids as energy sources. Amino acids regulate in vitro toxin production and support colonization of C. difficile in antibiotic-treated mice. Dysbiotic microbial communities showed significantly decreased expression of multiple genes related to amino acid uptake and metabolism, resulting in increased concentrations of 12 amino acids, with proline showing significant differences when compared to healthy mice microbiota. The ability to utilize proline provides a competitive advantage to C. difficile in germ-free mice transplanted with healthy-like and dysbiotic human stool consortia. Fecal microbiota transplant reduced free proline and decreased CDI susceptibility in dysbiotic mice (Mooyottu et al., 2017).

Recent studies have found increased indole levels (tryptophan metabolite involved in microbial growth, virulence induction, acid resistance, biofilm development) in the intestinal lumen of CDI patients, suggesting that C. difficile, which cannot produce this metabolite itself, would stimulate the production of indole by other bacteria to stop the growth and the development of indole-sensitive strains, including protective gut microbiota representatives, thus ensuring an intestinal environment conducive to its survival (Darkoh et al., 2019).

A recent study evaluated the relationship between the composition of the intestinal microbiota and level of fecal calprotectin in C. difficile asymptomatic and symptomatic patients. The asymptomatic patients have shown a modified microbiota, comparatively with the non-colonized patients, harboring significantly lower levels of Ruminococcaceae, Bilophila, Blautia, Faecalibacterium, Ruminococcus, and Sutterella, and higher levels of Enterobacteriaceae. In symptomatic patients the main deviations of gut microbiota were represented by higher levels of Bacteroides and lower levels of Blautia, Phascolarctobacterium, Prevotella, and Succinivibrio. These gut microbiota changes in symptomatic patients were accompanied by significantly higher levels of fecal calprotectin, comparatively with asymptomatic patients and controls. These data suggest that association of microbiota and inflammatory markers could be used to differentiate C. difficile colonization (CDC) from CDI (Han et al., 2020).

Knowledge regarding the gut microbiota in C. difficile colonized patients may elucidate the mechanisms that allow for colonization whilst protecting against infection (Crobach et al., 2020). To this end, a recent study by Crobach et al. (2020) analyzed the bacterial signatures associated with resistance and susceptibility to CDC and CDI. Both CDC and CDI were associated with decreased gut microbial diversity and differences in the relative abundance of taxa such as Lachnospiraceae, Ruminococcaceae, Fusobacteriaceae, Bacteroides, Veillonella, and Eubacterium hallii (Crobach et al., 2020).

INFANT GUT MICROBIOTA CHARACTERISTICS AND POSSIBLE EXPLANATIONS FOR THE LOW PATHOGENICITY OF C. DIFFICILE IN NEONATES

During childhood, the intestinal microbiota is subject to many factors that shape the microbiota on short- and long-term. Apart from the maternal-fetal transmission of certain bacterial components, the microbiota is influenced by the type and the time of birth, the place of birth (hospitals or home births), the type of feeding (breastfeeding or artificial feeding), administration of probiotic and prebiotic supplements, dietary factors, antibiotics and other drugs, sex, and other genetic differences and environmental factors, such as exposure to pets, number of family members, rural or urban environment, hygiene, geographical factors (Combellick et al., 2018; Mohammadkhah et al., 2018; Akagawa et al., 2019).

Taking into account the numerous factors that could influence the intestinal microbiota of the newborns, the healthy profile of this age group is considered to be represented by the types of gut bacteria and the abundances found in vaginally delivered, exclusively breastfed and not exposed to antibiotics neonates (Arboleya et al., 2015; Combellick et al., 2018; Mohammadkhah et al., 2018; Akagawa et al., 2019). It has been hypothesized that bacterial colonization of the digestive tract begins in utero, the healthy placenta bearing
a low biomass microbiome, composed of non-pathogenic species belonging to the Tenericutes, Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria phyla, while in the amniotic fluid predominate Proteobacteria. However, the results reported by different studies are contradictory and depend on the sampling method and the culture-based or molecular-based approaches. Recent metagenomic studies conclude that the placenta does not harbor a specific, consistent and functional microbiota (Gschwind et al., 2020). The meconium harbors a cultivable microbiota, initially dominated by Bacteroides-Prevotella. The digestive contamination of the fetus occurs most probably by swallowing the amniotic fluid, starting with the 10th week after conception (Chong et al., 2018). The transfer of Enterococcus faecium from pregnant female mice into meconium was also demonstrated experimentally (Jimenez et al., 2008). All these lead to the conclusion that changes in the maternal internal environment may affect both fetal and newborn development (Zhuang et al., 2019).

**C. difficile Colonization in Infants**

Neonates are uniquely susceptible to *C. difficile* colonization because of the neonatal intestine’s immaturity and intestinal microbiota instability (Lees et al., 2016). The main source of colonization seems to be the environmental exposure to *C. difficile* spores within the nursery or healthcare environment rather than the mother, the rates of *C. difficile* detection increasing with the length of stay in these units.

In infants <1 month of age, *C. difficile* has an average colonization rate of 37%, ranging between 0 and 61%. Between 1 and 6 months of age, the colonization rate is still high at 30% and drops to about 10% by the end of the first year of life (Kuiper et al., 2017). However, the colonization rates reported by different studies vary from 14 to 71% in children <12 months of age. This age group is most commonly colonized with non-toxigenic strains and they are asymptomatic (Khalaf et al., 2012). The asymptomatic carriage rate continues to drop until about 3 years of age, when it stabilizes to carriage rates of 0–3%, similar to those found in adults, together with a progressive raise in serum IgG antibody concentrations against toxins A and B between birth and 24 months of age (Antonara and Leber, 2016). Around 3 years of age, the intestinal microbiota of the child is stabilized, acquiring the characteristics of the adult microbiota, which might explain the increase of symptomatic CDI starting with this age.

Moreover, other studies are assuming that asymptomatic carriage of *C. difficile* is common in the young individuals of many other species, including dogs, pigs, and cattle (Deng and Swanson, 2015). In puppies, the association between lower bacterial community diversity and *C. difficile* colonization was statistically significant, and certain bacterial taxa were preferentially associated with *C. difficile* colonization (Berry et al., 2019). Similar associations have also been found in human studies. Unweaned puppies that were not colonized with *C. difficile* had higher relative abundance of taxa from the...
clostridia genera than unweaned puppies that were colonized with *C. difficile* (Berry et al., 2019). Many studies have found higher colonization rates with *C. difficile* in formula-fed infants than in breastfed infants (Cooperstock et al., 1983; Jangi and Lamont, 2010). Also, the breastfed infants colonized by *C. difficile* had significantly lower colony counts than formula-fed infants, probably because the human colostrum contains neutralizing antibodies to toxins A and B (Rolfe and Song, 1995; Jangi and Lamont, 2010).

There are no studies comparing *C. difficile* carriage rate regarding the delivery mode, but it might have persistent effects on microbiota beyond infancy. The lack of "bacterial baptism" of vaginal birth or other confounding factors associated with cesarean delivery, as well as maternal obesity, antibiotic administration, gestational age, and breastfeeding pattern, could influence the *C. difficile* carriage rate (Kyne et al., 2000; Liu et al., 2019). However, there were found to be a significantly higher number of Clostridia in the stool of children vaginally delivered (VD) than in those delivered by C-section (CS) (Reyman et al., 2019). No association was found with prematurity as a risk factor for *C. difficile* infection (Lees et al., 2016).

The question arises whether the newborns and adults, which are asymptomatic carriers of *C. difficile*, might have a particular gut microbiota composition that allows colonization to occur without any clinical manifestations. Lack of disease has also been related to immature or diminished receptor sites for toxin A along the intestinal epithelium (Keel and Songer, 2007). In exchange, CDI may be more likely to manifest in certain populations of infants harboring pathological intestinal conditions, such as those with Hirschsprung’s disease, all these demonstrating the link between *C. difficile* colonization and intestinal homeostasis (Sammons et al., 2013).

In conclusion, the high carriage rate of *C. difficile* colonization in neonates can be explained by the immaturity of the neonatal intestine and the presence of a less complex intestinal microbiota, as compared to adults. However, postnatal microbial species, together with the lack of receptors for clostridial toxins protect help babies from the deleterious effects of *C. difficile* toxins.

**Neonate Gut Microbiota Signatures Associated With *C. difficile* Colonization**

Considering that *C. difficile* only occasionally produces clinical manifestations in infants, one can state that a specific microbiota composition probably consolidating a specific environment in newborns helps protect babies from the deleterious effects of *C. difficile* toxins, which occur in dysbiotic adults.

The newborn microbiota is dominated by Gram-positive cocci, *Enterobacteriaceae* or *Bifidobacteriaceae*, with a sequential transition to a microbiota dominated by *Bifidobacteriaceae*. It is well known that *Bifidobacteria* upregulate IL-10 production by intestinal dendritic cells explaining the lack of clinical symptoms in infants colonized with *C. difficile* (Huurre et al., 2008). In CS neonates, decreased levels of T cells and CD4+ helper T cells were noticed, probably due to the failure of the immature infant immune system to activate an inflammatory response (Jangi and Lamont, 2010; Collins and Auchtung, 2017; Francino, 2018).

Prematurity might not be a risk factor for *C. difficile* infection (Lees et al., 2016), probably due to the fact that in general, the intestinal microbiota of the premature child is dominated by Proteobacteria, even if breastfed, and the species of *Clostridium* and *Veillonella* appear later. However, the microbiota of the premature infant is strongly influenced by pre- and postnatal antibiotic therapy (Staude et al., 2018). Hospitalization and antibiotic exposure induce indigenous microbiota imbalance (McFarland et al., 2016). Antibiotic treatment in neonates' intensive care units (NICU) was associated with a lower *C. difficile* colonization rate, but colonization with *C. difficile* occurred rapidly after cessation of antibiotics. In children in the NICU, born prematurely, the colonization with *Bifidobacteriaceae* is delayed.

Breast milk protects against infections in infants due to the presence of immunological factors such as immunoglobulin A (IgA), including neutralizing antibodies to *C. difficile* toxins A and B (Jangi and Lamont, 2010). *Ruminococcus* (which is more commonly found in the gut of breastfed infants) is thought to inhibit the growth of *Clostridia*, thereby preventing colonization by *C. difficile* (Morelli, 2008).

Increased levels of immunoglobulin-producing cells in peripheral blood have been observed in CS infants, probably due to excessive exposure to antigens at the level of the vulnerable intestinal barrier. In addition, breastfeeding contributes to the maturation of the infant’s immune system and modulates microbiota development. The microbiota of breastfed children is less diverse but contains more *Bifidobacterium* spp., also explaining the protection against deleterious pro-inflammatory responses triggered by CDI (Derrien et al., 2019). However, *Bifidobacteria* significantly decrease in abundance upon cessation of breastfeeding.

In the clinical cases of neonate necrotizing enterocolitis (NEC), a decrease of bacterial diversity and of *Bacteroidetes* phyla, as well as of *Bifidobacteria*, were observed, with the more frequent presence of potentially pathogenic organisms, such as *Staphylococcus aureus*, *Enterococcus*, *Escherichia coli*, *Shigella spp.*, *Citrobacter spp.*, *Klebsiella spp.* (Dobbler et al., 2017). Among the strictly anaerobic bacteria that have been associated with NEC, the majority belong to the *Clostridium* genus (e.g., *C. butyricum*, *C. neonatale*, *C. perfringens*, *C. paraputrificum*, and *C. difficile* have been associated with NEC in preterm neonates) (Zhou et al., 2015; Rozé et al., 2017; Schönherr-Helec et al., 2018; Schönherr-Helec and Aires, 2019). Moreover, a NEC-associated microbiota, such as *C. perfringens* has been identified in meconium samples (Heida et al., 2016).

**MANIPULATION OF GUT MICROBIOTA AS ADJUNCTIVE THERAPY OF *C. difficile* INFECTION**

The first step in *C. difficile* treatment is the de-escalation of antibiotic treatment. Depending on the degree of *C. difficile* risk induction, the antibiotics were divided into three groups: high (fluoroquinolones, 2nd and 3rd generation cephalosporins, clindamycin, ampicillin, broad-spectrum
TABLE 2 | Microbiota-centered therapeutic approaches with proven beneficial effects in CDI.

| Type of microbiota-targeted intervention | Administration methods | Effects | References |
|-----------------------------------------|------------------------|--------|-----------|
| Probiotics/prebiotics/synbiotics        |                        |        |           |
| Lactobacillus plantarum Induca          | Use of xylitol as symbiotic to enhance the probiotic engraftment and effects | Total inhibition of C. difficile spores germination in vitro; reduction of mouse mortality | Ratsep et al., 2017 |
| Bifidobacterium longum Bifidobacterium breve | Use oligo-fructosaccharides as a carbon source (symbiotic effect) | Reduction in toxicity | Valdes-Varela et al., 2016 |
| Bacillus clausii O/C                    | Administered alone     | Neutralization of C. difficile toxin inhibition of C. difficile toxins by bacterial secreted compounds (serine protease, M-protease) | Ripert et al., 2016; Aktories et al., 2017 |
| Bacillus thunbergiiis                   | Administered alone     | Production of bacteriocin direct inhibition | Mills et al., 2018 |
| Enterococcus durans                     | Administered alone and with bacteriocins (reuterin, nisin) | Production of durancin | Hanchi et al., 2017 |
| Lactobacillus reuteri                   | Administered alone     | Direct inhibition production of antibacterial substances such as reuterin obtained through fermentation of glycerol | Spinel et al., 2017 |
| Multi-strain capsule (Lactobacillus acidophilus NCGM, ATCC 700396; Lactobacillus paracasei Lpc-37, ATCC SD5275; Bifidobacterium lactis Bi-07, ATCC SC5220; B. lactis Bi-04, ATCC SD5219) | Administered as multi-strain capsule | Probiotic adjunct therapy was associated with a significant improvement in diarrhea outcomes | Barker et al., 2017 |
| Spores of Firmicutes phylum (e.g., SER-109, SER-262) | Administration of purified spores | Repopulation the gut microbiota | Khanna et al., 2016; Gnocchi et al., 2020 |
| Postbiotics                             | Administration of microbe-free fecal filtrates | Rapid shifts in gut microbial composition | Kelly et al., 2016; Hota et al., 2017; Ott et al., 2017 |
| Competition for resources               |                        |        |           |
| Non-toxigenic C. difficile (NTCD)       |                        |        |           |
| Fecal microbiota transplantation       |                        |        |           |
| Consortia of fecal bacteria quality-controlled and semi-standardized (e.g., RBK2660) | Use of fecal derivatives for the treatment of CDI | Repopulation of the gut microbiota | Orenstein et al., 2018 |

penicillins with inhibitors, except for ticarcillin with clavulanate, and piperacillin with tazobactam), moderate (macrolides, trimethoprim/sulfamethoxazole, other penicillins, and sulfonamides) and low risk (aminoglycosides, bacitracin, carbapenems, chloramphenicol, daptomycin, metronidazole, rifampicin, teicoplanin, tigecycline, tetracycline, and vancomycin) (Kukla et al., 2020). Current standard treatment for CDI involves treatment with antibiotics such as metronidazole, vancomycin, or fidaxomicin (Mills et al., 2018; Gnocchi et al., 2020). Vancomycin is the first-line antibiotic therapy for both first episode of infection and fulminant infections in adults (Esposito et al., 2015; Wang et al., 2020). Unfortunately, vancomycin is a strong disruptor of gut microbiota, while the rate of CDI recurrence after treatment cessation occurs in 20–30% of patients (Lessa et al., 2015; Winston and Theriot, 2016). On the other hand, metronidazole is used especially in the first episodes of mild acute CDI and less for severe disease because the concentrations in the colon become readily undetectable due to the fact that it is absorbed very quickly (Gnocchi et al., 2020). The rapid absorption from the gut is also reflected in a negligible effect on normal microbiota (Lessa et al., 2015; Chilton et al., 2018). Fidaxomicin can be used in C. difficile non-severe and also severe infections treatment due to the fact that it is poorly absorbed at the intestinal level, ensuring the persistence of killing concentration in the gut and has a narrow antimicrobial spectrum (e.g., Gram-positive and Gram-negative anaerobes and facultative aerobes) unafflicting the equilibrium of the normal intestinal microbiota (Tannock et al., 2010; Louie et al., 2011, 2012; Gnocchi et al., 2020). Moreover, in another study, vancomycin and metronidazole treatment, but not fidaxomicin were associated with the potentially pathogenic fungal operational taxonomic units’ emergence as well as with bacterial functions enriched for xenobiotic metabolism that could contribute to dysbiosis that could favor the occurrence, persistence and recurrence of CDI (Lamendella et al., 2018).

Adjuvatory therapies are frequently used due to the important role of gut microbiota disturbances in C. difficile pathogenesis (Table 1 and Figure 1). Thus, specific manipulation of the microbiota to ameliorate dysbiotic changes and restore intestinal microbiota homeostasis could represent an essential part of the therapy (Table 2).

One way to modulate microbiota is using “biotics” with beneficial impact on resident microbiota that confer health benefits for the host, such as probiotics (e.g., *Saccharomyces boulardii*, *Lactobacillus*, *Bifidobacterium*, and probiotic mixtures), prebiotics, symbiotics or postbiotics.
(Surawicz et al., 2000; Na and Kelly, 2011; Maziade et al., 2015; Collins and Auchtung, 2017). According to International Scientific Association for Probiotics and Prebiotics (ISAPP), prebiotics are substrates different from fibers, that are selectively metabolized by host microorganisms. Synbiotics are complementary/synergistic mixtures comprising live microorganisms (probiotics) and prebiotics. In case of synergistics synbiotics the two components taken individually do not have to meet criteria for prebiotic or probiotic. Postbiotics are cellular fractions or structures prepared from inactivated microbes. The postbiotic preparations exclude filtrates or live cultures individual components, while inactivated probiotics are not considered automatically postbiotics, unless a health benefit is demonstrated.

Most data regarding the protective role of commensal bacteria against C. difficile infection were obtained by studying the effects of different probiotics. For example, Bifidobacterium breve (YH68), widely used in the field of food fermentation and biomedicine, has shown antibacterial activity against C. difficile, by inhibiting the growth, spore production, toxigenesis and virulence gene expression (Valdes-Varela et al., 2016; Yang and Yang, 2019), potentiating the effect of anti-C. difficile antibiotics in vitro (Yang and Yang, 2018) or preventing the occurrence of clinical manifestations in vivo (Yun et al., 2017). Probiotic use has been shown to decrease CDI incidence in high-risk populations by as much as 50%, especially when they are combined with prebiotics (Shen et al., 2017).

It was demonstrated that the administration of non-living bacteria or microbial components (e.g., proteins, lipids, or nucleic acids) has an immunostimulatory effect proving that the beneficial impact on the host health is due to the physical interaction of specific microbial components, but in order to be effective for a long period, these need continuous administration.

Many bacterial strains such as Bacillus clausii and Lactobacillus reuteri have been shown to secrete soluble compounds that directly inhibit C. difficile (Khalaf et al., 2012; Deng and Swanson, 2015; Antonara and Leber, 2016). Organisms that produce secondary bile acids, such as Clostridium scindens, enhance C. difficile colonization resistance (Winston and Theriot, 2016).

Fecal microbiota transplantation (FMT) is considered the most effective microbiota-targeted intervention for the treatment of antibiotic-refractory CDI (Arbel et al., 2017), but however, the long-term effects, including the risk of other diseases, are not known (Schaffler and Breitruck, 2018).

Another therapeutic approach is the administration of non-toxigenic C. difficile strains or a mixture of spore-forming commensals, which act by providing nutritional niche competition. Despite their efficiency in decreasing CDI recurrence, there is the risk of switching to the toxigenic phenotype (Brouwer et al., 2013; Khanna et al., 2016).

**CONCLUSION**

The available studies suggest that C. difficile colonization and infection are influenced by the presence, absence or abundance of certain bacteria in the human gut, which could generate favorable conditions for germination, proliferation and production of clostridial toxins which, on their turn, will alter the integrity of the intestinal mucosa. Therefore, the clinical manifestations and severity of CDI are linked to gut dysbiosis, that could have multiple causes, among which the administration of high-risk antibiotics. The presence of protective microbial species, together with the particularities of the immune system and lack of receptors for clostridial toxins could explain the fact that in children, despite the high carriage rate, the symptomatic and severe cases are rare. However, if there is a gut microbiota composition predisposing to C. difficile asymptomatic carriage or clinical infection still needs clarification. Also, the mechanisms involved in C. difficile crosstalk with the commensal microbiota and/or particular soluble compounds remain only partially explained. Adjunctive microbiota-targeting therapies based on probiotics, prebiotics, postbiotics, synbiotics, non-toxigenic bacteria or fecal microbiota transplantation proved to be very useful for the therapeutic management of CDI.

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All authors contributed equally to this manuscript with the conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

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Aktories, K., Schwab, C., and Jank, T. (2017). Clostridium difficile toxin biology. *Annu. Rev. Microbiol.* 71, 281–307.

Amrane, S., Hocquart, M., Afouda, P., Kuete, E., Pham, T. P., Dione, N., et al. (2019). Metagenomic and culturomic analysis of gut microbiota dysbiosis during Clostridium difficile infection. *Sci. Rep.* 9:12807. doi: 10.1038/s41598-019-49189-8

Antharam, V. C., Li, E. C., Ishmael, A., Sharma, A., Mai, V., Rand, K. H., et al. (2013). Intestinal dysbiosis and depletion of butyrogenic bacteria in Clostridium difficile infection. *Ann. Rev. Microbiol.* 71, 281–307.

**REFERENCES**

Abt, M. C., McKenney, P. T., and Pamer, E. G. (2016). *Clostridium difficile* colitis: pathogenesis and host defence. *Nat. Rev. Microbiol.* 14, 699–620. doi: 10.1038/nrmicro.2016.108

Akagawa, S., Tsuii, S., Onuma, C., Akagawa, Y., Yamaguchi, T., Yamagishi, M., et al. (2019). Effect of delivery mode and nutrition on gut microbiota in neonates. *Ann. Nutr. Metab.* 74, 132–139. doi: 10.1159/000496427

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difficultie infection and nosocomial diarrhea. *J. Clin. Microbiol.* 51, 2884–2892. doi: 10.1128/JCM.00845-13

Antonara, S., and Leber, A. L. (2016). Diagnosis of *Clostridium difficile* infections in children. *J. Clin. Microbiol.* 54, 1425–1433.

Arbel, L. T., Hsu, E., and McNally, K. (2017). Cost-effectiveness of fecal microbiota transplantation in the treatment of recurrent *Clostridium difficile* infection: a literature review. *Careu* 9:e1599.

Arboleya, S., Sanchez, B., Milani, C., Duranti, S., Solis, G., Fernandez, N., et al. (2015). Intestinal microbiota development in preterm neonates and effect of perinatal antibiotics. *J. Pediatr.* 166, 538–544. doi: 10.1016/j.jpeds.2014.09.041

Avni, T., Babitch, T., Ben-Zvi, H., Hijazi, R., Ayada, G., Atamna, A., et al. (2020). *Clostridium difficile* infection in immunocompromised hospitalized patients is associated with a high recurrence rate. *Int. J. Infect. Dis.* 90, 237–242. doi: 10.1016/j.ijid.2019.10.028

Barker, A. K., Duster, M., Valentine, S., Hess, T., Archbold-Pannone, L., Guer rant, R., et al. (2017). A randomized controlled trial of probiotics for *Clostridium difficile* infection in adults (PICO). *J. Antimicrob. Chemother.* 72, 3177–3180. doi: 10.1093/jac/dkx254

Battaglioli, E. J., Hale, V. L., Chen, J., Jeraldo, P., Ruiz-Mojica, C., Schmidt, B. A., et al. (2018). *Clostridium difficile* coinfects mice with mixed *Clostridium difficile* strain into toxin producers. *PloS One* 14:e0215497. doi: 10.1371/journal.pone.0215497

Borali, E., and De Giacomoni, C. (2016). *Clostridium difficile* infection in children: a review. *Pediatr. Gastroenterol. Nutr.* 65, e130–e140.

Britton, R. A., and Young, V. B. (2014). Role of the intestinal microbiota in resistance to colonization by *Clostridium difficile*. *Gastroenterology* 146, 1547–1553.

Brouwer, M. S., Roberts, A. P., Hussain, H., Williams, R. J., Allan, E., and Mullany, B. T., et al. (2014). Regime shifts, resilience, and biodiversity in ecosystem management. *Science* 313, 1719–1727. doi: 10.1126/science.1237439

Bush, J. E., Garcia, M., Ribble, L., and Crowell, K. (2011). Clinical inquiry: what risk factors contribute to *C difficile* diarrhea? *J. Family Pract.* 60, 545–547.

Ceballos-Baumann, A. O., and Schaefer, E. M. (2013). Long-term stability of the human gut microbiota. *Microbiol. Spectr.* 1:274. doi: 10.1128/mSystems.00346-18

Collins, J., and Auchtung, J. M. (2017). Control of *Clostridium difficile* infection by defined microbial communities. *Microbiol. Spectr.* 5, 1–25.

Conte, M., Riegler, L., Woodruff, C. W., and Onderdonk, A. (1983). Influence of age, sex, and diet on asymptomatic colonization of infants with *Clostridium difficile*. *J. Clin. Microbiol.* 17, 830–833. doi: 10.1128/17.5.830-833.1983

Crogh, E. S., T. M., et al. (2008). Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile* infection and nosocomial diarrhea. *J. Clin. Microbiol.* 46, 1547–1553. doi: 10.1128/JCM.00845-13

Derrien, M., Alvarez, A. S. Z., and de Vos, W. M. (2019). The gut microbiota in the first decade of life. *Trends Microbiol.* 27, 997–1010. doi: 10.1016/j.tim.2019.08.001

Dieterle, M. G., Rao, K., and Young, V. B. (2019). Novel therapies and preventative strategies for primary and recurrent *Clostridium difficile* infections. *Ann. N.Y. Acad. Sci.* 1435, 110–138. doi: 10.1111/nyas.13939

Dobler, P. T., Procianoy, R. S., Mai, V., Silveira, R. C., Corso, A. L., Rojas, B. S., et al. (2017). Low microbial diversity and abnormal microbial succession is associated with necrotizing enterocolitis in preterm infants. *Front. Microbiol.* 8:2243. doi: 10.3389/fmicb.2017.02243

Dogra, S. K., Dore, J., and Damak, S. (2020). Gut microbiota resilience: definition, link to health and strategies for intervention. *Front. Microbiol.* 11:572921. doi: 10.3389/fmicb.2020.572921

Dupont, H. L. (2011). Gastrointestinal infections and the development of irritable bowel syndrome. *Curr. Opin. Infect. Dis.* 24, 503–508. doi: 10.1097/QCO.0b013e32834a9662

Esposito, S., Umbrello, G., Castellazzi, L., and Principi, N. (2015). Treatment of *Clostridium difficile* infection in pediatric patients. *Exp. Rev. Gastroenterol. Hepatol.* 9, 747–755. doi: 10.1586/17474124.2015.1039988

Fagioli, S., Pragnell, M., and Cammarota, G. R. (2018). Microbiologic factors for *Clostridium difficile* severe infection in a newborn. *Arch. Dis. Child.*** 104, A311.

Fakhri, J. L., Felipe, J. S., Pal, L. P., da Silva, B. B., Correa, R. O., de Andrade, M. C. P., et al. (2019). Butyrate protects mice from *Clostridium difficile*-induced colitis through an HIF-1-dependent mechanism. *Cell Rep.* 27, 750–761. doi: 10.1016/j.celrep.2019.03.034

Faith, J. J., Guerje, J. L., Charbonneau, M., Subramanian, S., Seedorf, H., Goodman, A. L., et al. (2013). The long-term stability of the human gut microbiota. *Science* 340, 1237439.

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., et al. (2004). *Regime shifts, resilience, and biodiversity in ecosystem management.* *Annu. Rev. Ecol. Evol. Syst.* 35, 557–581.

Francisco, M. P. (2018). Birth mode-related differences in gut microbiota colonization and immune system development. *Ann. Nutr. Metab.* 73(Suppl. 3), 12–16. doi: 10.1159/000490842

Franzosa, E. A., Sirota-Madi, A., Avila-Pacheco, J., Fornellos, N., Kaiser, H. J., Reinker, S., et al. (2019). Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat. Microbiol.* 4, 293–305.

Gerding, D. N., Meyer, T., Lee, C., Cohen, S. H., Murthy, U. K., Poirier, A., et al. (1984). *Clostridium difficile* colonization and immune system development. *Ann. Intern. Med.* 100, A311.

Ghose, C. (2013). *Clostridium difficile* infection in the twenty-first century. *Emerg. Microbes Infect.* 2:e62. doi: 10.1038/emi.2013.62
Human Microbiome Project, C. (2012). Structure, function and diversity of Gnocchi, M., Gagliardi, M., Gismondi, P., Gaiani, F., De' Angelis, G. L., and Vasilescu et al. Gut Microbiota for Health: An Emerging Pathogen in Children. Dis. 14, 105–113.

Khan, S., Pardi, D. S., Kelly, C. R., Kraft, C. S., Dhere, T., Henn, M. R., et al. (2016). A novel microbiome therapeutic increases gut microbial diversity and prevents recurrent Clostridium difficile infection. J. Infect. Dis. 214, 173–181. doi: 10.1093/infdis/jiv766

Kho, Z. Y., and Lal, S. K. (2018). The Human gut microbiome – a potential controller of wellness and disease. Front. Microbiol. 9:3835. doi: 10.3389/fmicb.2018.03835

Khoruts, A., Dicksveld, J., Janson, J. K., and Sadowsky, M. J. (2010). Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea. J. Clin. Gastroenterol. 44, 354–360. doi: 10.1097/MCG.0b013e3181e75702

Kuiper, G. A., van Prehn, J., Ang, W., Kneepkens, F., van der Schoor, S., and de Meij, T. (2017). Clostridium difficile infections in young infants: case presentations and literature review. IDCases 10, 7–11. doi: 10.116/j.idc.2017.07.005

Kukla, M., Adrych, K., Dobrowolska, A., Mach, T., Regula, J., and Rydzewska, G. (2020). Guidelines for Clostridium difficile infection in adults. Prz. Gastroenterol. 15, 1–21. doi: 10.5114/pg.2020.93629

Kyne, L., Warny, M., Qamar, A., and Kelly, C. P. (2008). Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. N. Engl. J. Med. 342, 390–397. doi: 10.1056/NEJM200803120340604

Lamendella, R., Wright, J. R., Hackman, J., McLimans, C., Toole, D. R., Bernard Rubio, W., et al. (2018). Antibiotic treatments for Clostridium difficile infection are associated with distinct bacterial and fungal community structures. mSphere 3, e00572-17. doi: 10.1128/mSphere.00572-17

Lawler, A. J., Lamb, P. A., and Worthington, T. (2020). A revised understanding of Clostridioides difficile spore germination. Trends Microbiol. 28, 744–752. doi: 10.1016/j.tim.2020.03.004

Lazar, V., Ditu, L., Pricaliboru, G. G., Gheorghe, I., Curutiu, C., Holban, A. M., et al. (2018). Aspects of gut microbiota and immune system interactions in infectious diseases, immunopathology, and cancer. Front. Immunol. 9:1830. doi: 10.3389/fimmu.2018.01830

Lazar, V., Ditu, L., Pricaliboru, G. G., Picu, A., Petcu, L., Cucu, N., et al. (2019). Gut microbiota, host organism, and diet triatogue in diabetes and obesity. Front. Nutr. 6:212. doi: 10.3389/fnut.2019.00021

Lees, E. A., Miyajima, F., Pirromohamed, M., and Carrol, E. D. (2016). The role of Clostridium difficile in the paediatric and neonatal gut - a narrative review. Eur. J. Clin. Microbiol. Infect. Dis. 35, 1047–1057. doi: 10.1007/s10096-016-2639-3

Lessa, F. C., Mu, Y., Bamberg, W. M., Beldavs, Z. G., Dumyati, G. K., Dunn, J. R., et al. (2015). Burden of Clostridium difficile infection in the United States. N. Engl. J. Med. 372, 825–834.

Levine, J. M., and d’Antonio, C. M. (1999). Elton revisited: a review of evidence linking diversity and invisibility. Oikos 87, 15–26.

Liu, Y., Qin, S., Song, Y., Feng, Y., Li, N., Xue, Y., et al. (2019). The perturbation of infant gut microbiota caused by cesarean delivery is partially restored by exclusive breastfeeding. Front. Microbiol. 10:598. doi: 10.3389/fmicb.2019.00598

Lo Presti, A., Zorzi, F., Del Chierico, F., Altomore, A., Cocca, S., Avola, A., et al. (2019). Fecal and mucosal microbiota profiling in irritable bowel syndrome and inflammatory bowel disease. Front. Microbiol. 10:1655. doi: 10.3389/fmicb.2019.01655

Louie, T. J., Cannon, K., Byrne, B., Emery, J., Ward, L., Eyben, M., et al. (2012). Fidaxomicin preserves the intestinal microbiome during and after treatment of
Clostridium difficile infection (CDI) and reduces both toxin reexpression and recurrence of CDI. Clin. Infect. Dis. 55(Suppl. 2), S132–S142. doi: 10.1093/cid/cis338

Louie, T. J., Miller, M. A., Mullane, K. M., Weiss, K., Lentnek, A., Golany, Y., et al. (2011). Fidaxomicin versus vancomycin for Clostridium difficile infection. N. Engl. J. Med. 364, 422–431.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., and Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. Nature 489, 220–230. doi: 10.1038/nature11550

Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., et al. (2016). Gut microbiota composition and pathogen dynamics in a murine model of Clostridium difficile infection. Anaerobe 42(Suppl.), S154–S158. doi: 10.1016/j.an errobe.2016.11.005

McFarland, L. V., Vizen, M., Dinleczy, E. C., and Goh, S. (2016). Comparison of pediatric and adult antibiotic-associated diarrhea and Clostridium difficile infections. World J. Gastroenterol. 22, 3078–3104. doi: 10.3748/wjg.v22.i11.3078

Milani, C., Ticinesi, A., Gerritsen, J., Nouvenne, A., Lugli, G. B., Schneeman, O. B., Hutenhower, C., Verbeke, K., et al. (2019). Establishing what constitutes a healthy human gut microbiome: state of the science, regulatory considerations, and future directions. J. Nutr. 149, 1882–1895. doi: 10.1093/jn/nxz154

McDonald, L. C., Gerding, D. N., Johnson, S., Bakken, J. S., Carroll, K. C., Coffin, S. E., et al. (2018). Clinical practice guidelines for Clostridium difficile infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin. Infect. Dis. 66, e1–e48.

McBurney, M. I., Davis, C., Fraser, C. M., Schneeman, O. B., Buttenhower, C., Verbeke, K., et al. (2019). Establishing what constitutes a healthy human gut microbiome: state of the science, regulatory considerations, and future directions. J. Nutr. 149, 1882–1895. doi: 10.1093/jn/nxz154

Perez-Cobas, A. E., Moya, A., Gosalbes, M. J., and Latorre, A. (2015). Colonization resistance of the gut microbiota against Clostridium difficile. Antibiotics 4, 337–357.

Perry, S., and Blekhman, R. (2019). Population dynamics of the human gut microbiome: change is the only constant. Genome Biol. 20.150. doi: 1186/s13059-019-1775-3

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59–65. doi: 10.1038/nature08821

Rajilic-Stojanovic, M., Shanahan, F., Guarrner, F., and de Vos, W. M. (2013). Phylogenetic analysis of dysbiosis in ulcerative colitis during remission. Inflamm. Bowel Dis. 19, 481–488. doi: 10.1097/MIB.0b013e31827c6edc

Ratsep, M., Koljalg, S., Sepp, I., Smidt, L., Truusalu, K., Songisepp, E., et al. (2020). A combination of the probiotic and prebiotic product can prevent the germination of Clostridium difficile spores and infection. Anaerobe 47, 94–103. doi: 10.1016/j.an aero.2017.03.019

Raymond, F., Ouaemur, A. A., Deraspe, M., Ishqal, N., Gregars, H., Dridi, B., et al. (2016). The initial state of the human gut microbiome determines its reshaping by antibiotics. ISME J. 10, 707–720. doi: 10.1038/ismej.2015.148

Rea, M. C., O’Sullivan, O., Shanahan, F., O’Toole, P. W., Stanton, C., Ross, R. P., et al. (2012). Clostridium difficile carriage in elderly subjects and associated changes in the intestinal microbiota. J. Clin. Microbiol. 50, 867–875. doi: 10.1128/JCM.01176-11

Reeves, A. E., Koenigsknecht, M. J., Bergin, I. L., and Young, V. B. (2012). Suppression of Clostridium difficile in the gastrointestinal tracts of germfree mice inoculated with a murine isolate from the family Lachnospiraceae. Infect. Immun. 80, 3768–3774. doi: 10.1128/IAI.00647-12

Reeves, A. E., Thériot, C. M., Bergin, I. L., Huffnagle, G. B., Schloss, P. D., and Young, V. B. (2011). The interplay between microbiome dynamics and pathogen dynamics in a murine model of Clostridium difficile Infection. Gut Microbes 2, 145–158. doi: 10.4161/gmic.2.3.16333

Ridlon, J. M., Kang, D. J., Hylemon, P. B., and Bajaj, J. S. (2014). Bile acids and the gut microbiome. Curr. Opin. Gastroenterol. 30, 332–338.

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., et al. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 7:14. doi: 10.3390/microorganisms7010014

Ripert, G., Racedo, S. M., Elie, A. M., Jacquet, C., Bressollier, P., and Urdati, M. C. (2016). Secreted compounds of the probiotic Bacillus clausii strain O/C inhibit the cytotoxic effects induced by Clostridium difficile and Bacillus cereus toxins. Antimicrob. Agents Chemother. 60, 3445–3454. doi: 10.1128/AAC.02815-15

Rodriguez, C., Romero, E., Garrido-Sánchez, L., Alcain-Martínez, G., Andrade, R. J., Taminiau, B., et al. (2020). Microbiota insights in Clostridium difficile infection and inflammatory bowel disease. Gut Microbes 12:1725220.

Rodriguez, C., Taminiau, B., Korsak, N., Avesani, V., Van Broeck, J., Brach, P., et al. (2016). Longitudinal survey of Clostridium difficile presence and gut microbiota composition in a Belgian nursing home. BMC Microbiol. 16:229. doi: 10.1186/s12866-016-0848-7

Rolle, R. D., and Song, W. (1995). Immunoglobulin and non-immunoglobulin components of human milk inhibit Clostridium difficile toxin A-receptor binding. J. Med. Microbiol. 42, 10–19. doi: 10.1099/00222615-42-1-10

Ross, C. L., Spinder, J. K., and Savidge, T. C. (2016). Structural and functional changes within the gut microbiota and susceptibility to Clostridium difficile infection. Anaerobe 41, 37–43. doi: 10.1016/j.an aero.2016.05.006

Rousseau, C., Lemeé, L., Le Monnier, A., Poilane, I., Pons, J. L., and Collignon, A. (2011). Prevalence and diversity of Clostridium difficile strains in infants. J. Med. Microbiol. 60(Pt. 8), 1112–1118. doi: 10.1099/jm.0.029736-0

Rozé, J. C., Ancel, P. Y., Lepage, P., Martin-Marchand, L., Al Nabbanzi, Z., Delannoy, J. A., et al. (2017). Nutrition EPIPAGE 2 study group; EPIFLORE study group. Longitudinal survey of the human gut microbiota composition in children. JAMA Pediatr. 167, 567–573.
Sartelli, M., Di Bella, S., McFarland, L. V., Khanna, S., Furuya-Kanamori, L., Abuzeid, N., et al. (2019). 2019 update of the WSES guidelines for management of Clostridiodes (Clostridium) difficile infection in surgical patients. World J. Emerg. Surg. 14:8. doi: 10.1186/s13027-019-0228-3
Schaffer, H., and Breitbuch, A. (2018). Clostridium difficile - from colonization to infection. Front. Microbiol. 9:466. doi: 10.3389/fmicb.2018.00466
Schönherr-Hellec, S., and Aresi, J. (2019). Clostridia and necrotizing enterocolitis in preterm neonates. Anaerobe 58, 6–12. doi: 10.1016/j.anaero.2019.04.005

Vakili, B., Fateh, A., Asadzadeh Aghdaei, H., Sotoodehnejadnematalahi, F., and Spinler, J. K., Auchtung, J., Brown, A., Boonma, P., Oezguen, N., Ross, C. L., Tannock, G. W., Munro, K., Taylor, C., Lawley, B., Young, W., Byrne, B., Solomon, K. (2013). The host immune response to Clostridium difficile infection. J. Innate Immun. 4, 149–158. doi: 10.1159/000332946

Shen, N. T., Maw, A., Tmanova, L. L., Pino, A., Ancy, K., Crawford, C. V., et al. (2017). Timely use of probiotics in hospitalized adults prevents Clostridium difficile infection: a systematic review with meta-regression analysis. Gastroenterology 152, 1889–1900.e9. doi: 10.1053/j.gastro.2017.02.003

Smits, W. K., Lyra, D., Lacy, D. B., Wilcox, M. H., and Kuiper, E. J. (2016). Clostridium difficile infection. Nat. Rev. Dis. Primers 2:16020.

Solomon, K. (2013). The host immune response to Clostridium difficile infection. Ther. Adv. Infect. Dis. 1, 19–35. doi: 10.1177/2049936112472173

Staude, B., Oehmke, F., Lauer, T., Behnke, J., Gopel, W., Schloter, M., et al. (2018). Clostridia and necrotizing enterocolitis in preterm neonates. Anaerobe 58, 6–12. doi: 10.1016/j.anaero.2019.04.005

Seekatz, A. M., and Young, V. B. (2014). Antibiotic-induced alteration to the bowel microbiota of Clostridium difficile-infected patients than in preterm neonates. Anaerobe 58, 6–12. doi: 10.1016/j.anaero.2019.04.005

Wilson, B. C., Vatanen, T., Cutfield, W. S., and O’Sullivan, J. M. (2019). The superdonor phenomenon in fecal microbiota transplantation. Front. Cell. Infect. Microbiol. 9.2. doi: 10.3389/fcimb.2019.00002

Winston, J. A., and Theriot, C. M. (2016). Impact of microbial derived secondary bile acids on colonization resistance against Clostridium difficile in the gastrointestinal tract. Anaerobe 41, 44–50. doi: 10.1016/j.anaero.2016.05.003

Yang, J., and Yang, H. (2018). Effect of bifidobacterium breve in combination with different antibiotics on Clostridium difficile. Front. Microbiol. 9:2953. doi: 10.3389/fmicb.2018.02953

Yang, J., and Yang, H. (2019). Antibacterial activity of bifidobacterium breve against Clostridioides difficile. Front. Cell. Infect. Microbiol. 9:288. doi: 10.3389/fcimb.2019.00288

Yun, B., Song, M., Park, D. J., and Oh, S. (2017). Beneficial effect of Bifidobacterium longum ATCC 15707 on survival rate of Clostridium difficile infection in mice. Korean J. Food Sci. Anim. Resour. 37, 368–375. doi: 10.5851/kosfa.2017.37.3.368

Zhang, L., Chen, H., Zhang, S., Zhuang, J., Li, Q., and Feng, Z. (2019). Intestinal microbiota in nutrition and health. BMJ 367, l4345–l4345. doi: 10.3390/fmicb.2018.02953

Zhang, L., Dong, D., Jiang, C., Li, Z., Wang, X., and Peng, Y. (2015). Insight into the host immune response to Clostridium difficile infection. J. Immunol. 195, 6072–6079. doi: 10.4049/jimmunol.1500282

Zhuang, L., Chen, H., Zhang, S., Zhuang, J., Li, Q., and Feng, Z. (2019). Intestinal microbiota in early life and its implications on childhood health. Genomics Proteom. Bioinform. 17, 13–25. doi: 10.1016/j.gpb.2018.10.002

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