Chapter 12
Interactions of Pathogenic *Escherichia coli* with Gut Microbiota

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Chapter Summary The composition of gut microbiota (GM) plays a key role in the defense against pathogenic species of *Escherichia coli*, such as enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC). The symbiotic relationship between the commensal microbiota and the host can be interrupted when the microbial composition is altered. Two of the most common threats to the balance of the microbiota that lead to dysbiosis are infectious diseases and antibiotic treatment. Crosstalk between GM and enteric pathogens includes nutrients availability and alterations in the mucus layer or in the oxygen metabolism. Especially in the Americas, the existence of co-infections with other pathogenic microorganisms (bacteria, parasites, or viruses) is a very important condition that has an impact on the outcome of EHEC infections. The integration between "omics" techniques offers a unique opportunity to dissect the metabolic and cellular processes of microbiota and to determine the components involved in the crosstalk between the pathogen and the host. Using these tools, several reports have associated the higher incidence of EHEC infections in children with a lesser maturity and a lower diversity in the composition of GM compared to adults. This overview is intended to show how much has been studied about the net interactions between EHEC and GM. However, the mechanisms by which the GM changes in response to virulence

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factors, to enhance host defense or conversely to exacerbate multi-organ damage, merit further investigations.

12.1 Microbiota in Health and Disease

The human gut microbiota (GM) is diverse, dynamic, and complex, consisting of commensal bacteria, fungi, and viruses; and among them, bacteria, of which there are over 1000 different species, constitute the majority (approximately 10^{14}) including beneficial and protective bacterial species and others that could be potentially harmful (Rinninella et al. 2019). Crosstalk between the host and GM maintains the homeostatic balance keeping the gastrointestinal tract healthy. GM contributes to gut maturation and host nutrition. Regarding pathogen resistance (Curtis et al. 2014), GM prevents colonization by pathogens through different mechanisms, such as nutrients competition (Pacheco et al. 2012), bacteriocin production, cross inhibition by metabolic products, and modulation of immune response (Abt and Pamer 2014).

Firmicutes, Bacteroides, Actinobacteria, and *Proteobacteria* phyla are the largest components of the normal GM. The imbalance in the qualitative and quantitative composition of microbiota is known as dysbiosis and may be a consequence of intestinal pathogenic processes such as infections or local autoimmunity and systemic disorders such as diabetes, obesity, Alzheimer's, Parkinson's, and hypertension, among others (Yu et al. 2014; Kim 2015; Chen et al. 2021; Jia et al. 2021). But reciprocally, changes in GM due to age (Claesson et al. 2011; Ringel-Kulka et al. 2013), diet alterations (Zimmer et al. 2012), ethnicity and geographic location (Prideaux et al. 2013), or inflammatory diseases could increase susceptibility to intestine colonization by specific pathogens (Zumbrun et al. 2013; Hall et al. 2018). The concept indicating that the composition of GM plays a key role in the defense against pathogenic species of *E. coli* is supported by an extensive bibliography (Lee et al. 2021).

The development, establishment, and dynamic changes of the human GM during life are not well-understood. Many factors can affect the composition of the infant's gut including mode of childbirth delivery, breastfeeding versus formula feeding (Penders et al. 2006; Jakobsson et al. 2014), and genetics (Yatsunenko et al. 2012). Furthermore, such differences in gut microbial colonization lead to differing gut pH levels, nutrient availability, and the presence of metabolites and antimicrobial compounds (Stecher and Hardt 2011). Although numerous studies have investigated the composition of the intestinal microbiota in infants and adults, it is currently unknown at what age the infant's microbiota is established and reaches an adult-like profile. Facultative bacteria such as *Escherichia*, *Enterococcus*, α-hemolytic *Streptococci*, and *Staphylococcus* species have been found to colonize the gastrointestinal (GI) tract of infants during their first days after birth, followed by colonization of anaerobic bacteria including *Bacteroides*, *Bifidobacterium*, and *Clostridium* species, due to anaerobic conditions and human milk oligosaccharides (Johnson and Versalovic

2012). Typically, the healthy GM is composed of only a minor proportion of the *Proteobacteria* phylum, and thus, a high abundance of these bacteria is often a sign of dysbiosis (Shin et al. 2015). The symbiotic relationship between the commensal microbiota and the host can be interrupted when the microbial composition is altered. Dysbiosis may be associated with either external factors (antibiotic treatment or diet) or internal factors (altered immune function, genetic susceptibility, and intestinal diseases such as recurrent infection with *Clostridioides difficile*, irritable bowel syndrome, colorectal cancer, ulcerative colitis, and Crohn's disease). In either case, changes in the composition and abundance of microorganisms alter the environment of the intestinal tract (Saeed et al. 2022).

In this regard, two of the most common threats to the balance of the microbiota that lead to dysbiosis are infectious diseases and antibiotic treatment. Previous reviews have outlined in detail the association between the presence and absence of specific microbiota species and GI infections (Kitamoto et al. 2016; Saeed et al. 2022). In particular, enteric pathogens have the ability to successfully overcome and change the composition of the resident microbiota in healthy individuals. On the other hand, a large body of evidence has demonstrated that antibiotics severely affect and/or eliminate the GM (protozoa and/or bacteria) (Rosengaus et al. 2011). Broad-spectrum antibiotics alter the microbiome by reducing diversity and shifting community composition (Pifer and Sperandio 2014). Although most of the microbiota return after treatment is ceased, some members of this community are lost and take a long time to recover. The consequent misbalance leads to changes in the metabolic profiles of the intestine, decreasing concentrations of amino acids and short-chain fatty acids (SCFAs) and increasing oligosaccharide levels.

12.1.1 Crosstalk Between Gut Microbiota and Enteric Pathogens

12.1.1.1 Nutrients Availability

Certain commensals directly exacerbate infection by attaching and effacing (A/E) pathogens, such as enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC), influencing the GI metabolic landscape. *Bacteroides thetaiotaomicron*, a major constituent of the human GM, encodes several glycoside hydrolases and polysaccharide lyases that contribute to the availability of nutrients within the intestine (Sonnenburg et al. 2005). Within the intestine, *B. thetaiotaomicron* is one of the prominent species in the *Bacteroidetes* phylum and degrades complex polysaccharides into monosaccharides that can be used by non-glycophagic bacterial species such as *E. coli* and *Citrobacter rodentium* (Sonnenburg et al. 2005). Fluctuations in sugar concentrations modulate virulence gene expression and colonization of the human pathogen EHEC (Njoroge et al. 2012). Microbiota-produced succinate has been shown to augment *C. difficile* expansion in mice (Ferreyra et al. 2014) and to disrupt the innate immune killing of *E. coli* by decreasing macrophage and

neutrophil function (Rotstein et al. 1989). Succinate is then sensed by EHEC at the epithelial lining, to induce locus enterocyte effacement (LEE) expression such as the EspA, a component of the type 3 secretion system (T3SS) (Ferreyra et al. 2014).

In addition, the major source of carbon in the colon is the mucus, which is decorated with sugars such as fucose. EHEC senses the fucose released from the mucus by *B. thetaiotaomicron* through the two-component system FusKR. FusKR modulates EHEC's metabolism to optimize its growth and decrease competition for carbon sources with the commensal *E. coli* (Pacheco et al. 2012). Another important metabolite found in the colon is indole, which is produced by microbiota-derived tryptophanase, the enzyme that catalyzes L-tryptophan conversion to indole. Indole is also known to be absorbed by host cells and helps strengthen the integrity of the intestinal barrier (Bansal et al. 2010). Both *E. coli* and *B. thetaiotaomicron* have the capacity to produce indole. It has been recently demonstrated that decreased indole concentrations promote bacterial pathogenesis, while increased levels of indole decrease bacterial virulence gene expression (Kumara and Sperandio 2019).

Biotin is an essential vitamin that humans cannot synthesize but acquire from their diet and microbiota. Under low biotin conditions in the colon, the preferred niche for EHEC, the global iron regulator Fur is no longer suppressed and proceeds to activate the LEE genes, promoting EHEC adherence. Significantly, dietary supplementation of biotin was enough to reduce adherence of EHEC in mice (Yang et al. 2015).

One of the multiple beneficial properties provided by the microbiota is the digestion of complex carbohydrates that are not hydrolyzed and absorbed in the small intestine, a process that is critical for gut homeostasis. SCFA produced from starches within the colon is mediated by *Firmicutes*, such as *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Bacteroides* spp. Acetate, propionate, and butyrate are the physiologically most abundant SCFAs found in the intestine. SCFAs are present in the colon in millimolar concentrations and in lower concentrations in the upper GI tract. EHEC uses these molecules to control the expression of virulence genes (Pifer and Sperandio 2014). In addition, the presence of mucin-derived carbon sources such as galacturonic acid or mannose has also been shown to induce virulence genes in EHEC (Carlson-Banning and Sperandio 2016). Altogether, several metabolites have been implicated in the crosstalk between the microbiota and the targeted enteropathogens. This knowledge should open new avenues in the development of new strategies to protect humans from these pathogens.

12.1.1.2 Mucus Barrier

The colonic mucus layer that overlies the gut epithelium is one of the first lines of defense against both commensal and pathogens and a reservoir of antimicrobial peptides and immunoglobulins. It is formed by an inner layer, which is tightly adhered to the epithelium and is poorly colonized by commensal bacteria, and an outer layer which contains host enzymes and metabolically different commensals, such as *Akkermansia muciniphila* (Li et al. 2015). Because of a fiber-deficient diet, the microbiota loses a major energy source and must consume mucus glycoproteins,

leading to the degradation of the mucus layer (Desai et al. 2016). In a gnotobiotic mouse model, it has recently been demonstrated that erosion of the mucus layer favors *C. rodentium* access to the colonic epithelium and leads to inflammation and lethal colitis (Desai et al. 2016). The impact of mucus degradation on increasing pathogen susceptibility in this study was like a previous report in mice knockout for Muc-2 (Bergstrom et al. 2010). In this context, it is worth noting that higher levels of mucolytic bacteria have been found in patients with inflammatory bowel disease (IBD).

During an established infection, EHEC and EPEC also influence gut motility and luminal flow by inducing osmotic diarrhea that temporarily removes the protective mucus barrier, providing pathogens and commensals direct access to the epithelium. Osmotic stress also causes microbiota perturbations, decreasing highly abundant taxa, such as *Bacteroidales* family *S24–7*, to the point of extinction while allowing for blooms of less prevalent taxa, with long-lasting effects on host health (Tropini et al. 2018).

A/E-producing pathogens also induce intestinal hyperplasia for their benefit. A study of *C. rodentium* infection showed that the hyperproliferation of crypt stem cells results in epithelial oxygenation, which favors aerobic pathogen expansion. In healthy crypts, mature colonocytes consume oxygen via oxidative phosphorylation, maintaining mucosal surface hypoxia. Hyperproliferation leads to the displacement of mature colonocytes by immature, undifferentiated cells, which have an altered metabolism toward anaerobic glycolysis and do not consume oxygen, which increases colonic oxygen (Lopez et al. 2016). The EPEC effector NleB has been shown to directly mediate cellular oxygen homeostasis and glucose metabolism by placing an N-acetylglucosamine moiety on HIF-1α, a master regulator of host cellular oxygen homeostasis, enhancing its transcriptional activity. Altered HIF-1α activity further facilitates the switch to increased glycolysis as opposed to oxidative phosphorylation (Xu et al. 2018).

12.1.2 Co-infections

The context in which a pathogen encounters the host environment can have an enormous impact on the outcome of the infection. In this context, another important aspect to consider, especially in the Americas, is the existence of co-infections with other pathogenic microorganisms, such as bacteria, parasites, or viruses. Dysbiosis due to such infections could dramatically affect the prognosis of A/E infections or even long-lasting diseases. Mathew et al. observed that children suffering from mixed viral-bacterial infections with either rotavirus or norovirus, combined with enteroaggregative *E. coli* (EAEC) or EPEC, had significantly increased diarrheal disease severity scores compared with children with viral infection only or mixed infection with EPEC, and the clinical conditions of the children were worsened with both pathogenic *E.coli* co-infections (Mathew et al. 2019). In this regard, the increasing incidence of pediatric EAEC infections has recently been reported in Argentina and it is concerning the detection of EAEC genes in bloody diarrhea or

hemolytic uremic syndrome (HUS). How EAEC long-lasting colonization of the infant gut affects the susceptibility to EHEC or Shiga toxin-producing *E. coli* (STEC) pathogenesis is an area of increasing attention that deserves further in-depth investigation.

Syndromic diagnostic techniques for gastrointestinal infections are increasingly used in Latin American countries. Due to the COVID-19 pandemic, Latin American countries had to make significant efforts to implement molecular diagnostic laboratories to support test-trace-isolate strategies. The increase in installed diagnostic capabilities has opened a new horizon for the use of syndromic diagnosis in these countries, improving diagnostic for several diseases, including infectious diarrhea. Although these techniques offer many advantages for the clinical management of patients, the impact of co-detection of diarrheagenic *E. coli* (DEC) pathogens needs to be addressed.

In a study conducted in Chile, using a molecular diagnostic technique for 22 gastrointestinal pathogens in 427 samples from children under 5 years of age with diarrhea, it was found that 87% of the samples have at least one enteropathogen. Interestingly, in 59% of the samples, the detection of at least two pathogens was found, with EPEC and EAEC strains being the pathogens found in the greatest quantity (over 90%) (Poulain et al. 2021). The high number of positive samples for more than one pathogen has been described in other Latin American countries and the rest of the world. In Brazil, using a multiplex polymerase chain reaction (PCR) in a cross-sectional, age-matched case-control study of diarrhea in children aged 2–36 months, from six cities in Brazil's semiarid region, a higher proportion of co-detection was found. Again, EAEC and EPEC had the highest prevalence when children presented with two pathogens (Lima et al. 2018).

The detection of enteropathogens in stool samples from healthy people has been widely reported throughout the world (Merino et al. 2020). This situation has not only become a challenge for the use and interpretation of syndromic tests in diarrhea but has also allowed to propose that enteric pathogens require host conditions that would trigger the expression of virulence factors in these enteropathogens, promoting the development of infectious symptoms. Among these factors, several research groups have investigated the role of the intestinal microbiota as a regulator of the virulence of enteropathogens.

12.1.3 Omics Tools for a More Comprehensive View of the Molecular and Physiological Events Underlying Diarrheal Disease

Human microbiota constitutes 90% of the total number of cells associated with our bodies, with only the remaining 10% comprised of human cells (Tojo et al. 2014). For years, culture techniques and biochemical characterization were the gold standard methods for identifying bacterial species, resulting in a research bias against

those microorganisms that could not be grown under laboratory conditions. However, the development of sequencing techniques has added valuable information as to the full establishment of the microbiota. All these techniques are based on polymorphisms found in the gene sequence coding for the small subunit ribosomal RNA (16S rRNA). The next generation sequencing (NGS) platform has facilitated the quantitative, phylogenetic identification of microbiota at various taxonomic levels (Fraher et al. 2012). The analysis of 16S rRNA gene sequences based on the percentage of their identity, using the operational taxonomic units (OTUs) or operational phylogenetic units (OPUs) for taxonomic assignment, has facilitated the identification of known and unknown microorganisms (Yarza et al. 2014).

Although the use of 16S rRNA gene sequencing has been the technique of choice for the study of the microbiota, the use of other "omics" techniques has allowed for deepening the knowledge of the interaction between pathogen-host and thus proposed mechanisms involved in the expression of virulence factors that control infection. One of the limitations of 16S rRNA gene sequencing is the precision to identify the microorganisms that compose it at the species level. Most 16S rRNA gene sequencing platforms allow resolution to the genus level, which makes it difficult to analyze the role of specific species in a pathogenic process. The use of metagenomics techniques, such as whole genome shotgun genomics, allows us not only to determine the microorganisms present in a sample at the species level but also to infer the functional abilities of these microorganisms (Boudar et al. 2022). In the case of diarrheal infections, the use of whole genome shotgun metagenomics has allowed the assembly of genomes of the species in the gut microbiota, the presence of antibiotic resistance genes and virulence factors, as well as the bacterial metabolic pathways that could be involved, considering the amount and type of bacterial species present (Wang et al. 2015). This information has been relevant for the study of outbreaks and identification of resistance markers that could compromise therapy in diarrheal infections (Hilt and Ferrieri 2022).

Regarding the identification of the genes involved in the metabolism of a particular bacterial community, the quantification of the expression of these genes (transcriptomics) and the identification of the metabolites (metabolome) in stool samples have had a strong impact in characterizing the intestinal environment associated to a particular infection (Hao et al. 2022). Both transcriptomics and metabolomics have made it possible to characterize the communities present in an infection, the expression of its genes, and the changes in the intestinal microenvironment in a pathogenic process (Tanaka et al. 2022). The integration between "omics" techniques offers a unique opportunity to dissect the metabolic and cellular processes that occur inside and outside the microorganisms that make up the microbiota and to determine the components involved in the crosstalk between the pathogen and the host.

12.1.4 Microbiota Changes During DEC Infections

Using the tools mentioned above, it has been determined that gut bacterial communities evolve into an adult-like community within the first years of life, and that during this period, interpersonal variations are significantly higher as compared to adults (Roswall et al. 2021; Yatsunenko et al. 2012). From birth, the dominant genus within the microbiome is *Bifidobacterium*, followed by *Bacteroides* and *Enterobacteriaceae*, while in adulthood, the relative abundance of *Bifidobacterium* decreases, and the *Firmicutes* and *Bacteroidetes* phyla become dominant within the intestinal microbiota (Odamaki et al. 2016).

Considering that the expression of virulence factors in bacterial pathogens is strongly regulated by environmental conditions at the infection site, it is not surprising that microbiota composition may play a role in the regulation of pathogenic mechanisms. On the other hand, DEC pathotypes have evolved from commensal *E. coli*, acquiring virulence traits and sensing mechanisms to express virulence factors at the right time and place for the successful colonization of intestinal cells.

Expression of virulence genes is a highly regulated process, mediated by environmental conditions and/or bacterial regulators, which can induce or silence its expression. Under well-defined environmental conditions, the expression of virulence genes occurs at a specific site, allowing the bacteria to initiate the infection process (Kitamoto et al. 2016). However, DEC virulence gene expression is not completely understood, and most studies have focused on unraveling the molecular mechanism occurring inside the bacteria; meanwhile, little is known about the environmental factors that regulate pathogenesis at a specific time or place. Several have demonstrated that during DEC infections, significant changes occur in the composition of the gut microbiota and metabolome. At the phyla level, the main changes have been associated with an increase of *Proteobacteria* and *Bacteroides* and a reduction in the abundance of *Firmicutes*, with a decrease in alpha diversity (Gallardo et al. 2017; Kieser et al. 2018; Mizutani et al. 2021).

Several reports have associated the higher incidence of STEC infections in children (Loconsole et al. 2020) with a lesser maturity and a lower diversity in the composition of GM compared to adults (Ringel-Kulka et al. 2013). These differences have been observed in both mice and humans, and neonates often lack beneficial *Clostridiales* (Kim et al. 2017). As described above, EPEC and EHEC cause characteristic intestinal pathology inducing the typical attaching and effacing lesions. Due to the poor ability of EPEC and EHEC to infect mice, much of the in vivo studies on A/E pathogenesis have been done using the natural murine pathogen *C. rodentium*, which is related to both EPEC and EHEC and form A/E lesions in the murine large intestine. Experiments done in mice strains by transferring cecal content from resistant to susceptible to *C. rodentium* infection have demonstrated that microbial composition might define the fate of the GI infection (Collins et al. 2014). Similarly, adult gnotobiotic mice transferred with intestinal content from neonatal mice showed increased susceptibility to *Salmonella enterica* serovar Typhimurium and *C. rodentium* than those transferred with intestinal content from

adult mice only (Kim et al. 2017). Some reports have described alterations in the composition of GM in patients with STEC infections. Lower numbers of *Bifidobacterias* and *Clostridiales* were found in the feces of patients infected with STEC O26:H11 than in healthy subjects (Gigliucci et al. 2018). *Bifidobacteria* participate in the NF-κB and SOS signaling pathways in intestinal epithelial cell (IEC) lines by downregulating the mRNA levels of inflammatory cytokines in response to stimulation with intact bacterial cells or bacterial cell wall components, such as lipopolysaccharide (LPS) (Riedel et al. 2006). Moreover, *Bifidobacteria* have been reported to have protective efficacy in mice infected with EHEC O157:H7 (Yoshimura et al. 2010). Many studies have demonstrated that *Clostridium* species are probiotics that control the intestinal inflammatory response caused by LPS, suggesting that they have preventive and therapeutic effects on EHEC infection (Guo et al. 2020; Xiao et al. 2021; Takahashi et al. 2004).

12.1.4.1 Interactions Between Gut Microbiota and STEC

Enterohemorrhagic *E. coli*, a specific subset of STEC, is one of DEC that can cause from uncomplicated diarrhea to hemorrhagic colitis and life-threatening sequelae such as HUS. EHEC colonizes the epithelial cells lining the terminal ileum and colon, where it causes A/E lesions and produces the potent Shiga toxins (Newell and La Ragione 2018). To establish an infection, EHEC strains must modulate their gene expression to overcome the obstacles of resistance to colonization presented by the microbiota of the GI tract. In fact, the EHEC pathogenicity is the result of numerous interactions with either the gut microbial environment or/and their host. Reports have been trying to elucidate these interactions and provide knowledge concerning the mechanisms used by the pathogens to persist in their host and cause disease (Baumler and Sperandio 2016; Jubelin et al. 2018). Most studies have focused on the pathogenic roles of Stxs and accessor virulence factors as harmful substances capable of inducing cell death. The question raised is why individuals infected with the same STEC strain, encoding the same virulence factors, manifest varying degrees of disease severity. Many authors tried to answer this question by analyzing interactions between the pathogen and gut microbiota.

The major virulence factor Stx has an AB_5 structure. The B subunits are responsible for binding to the host receptor globotriaosylceramide that is found on various cell types but primarily on intestinal and renal endothelial cells. The A subunit has N-glycosidase activity that removes an adenine in the host cell rRNA. This cleavage inhibits protein synthesis and cell death (Bryan et al. 2015). There are described two distinct Shiga toxin types, each one with the following subtypes: Stx1(Stx1a, Stx1c, and Stx1d) and Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, Stx2g, Stx2h, and Stx2i) (Scheutz et al. 2012; Bai et al. 2018; Lacher et al. 2016). In general, infections with Stx2a- and Stx2d-producing *E. coli* cause more severe disease than the others and are associated with higher rates of HUS (FAO WHO STEC Expert Group 2019).

Stx1 and Stx2 are encoded on lambdoid bacteriophages (LB). After bacterial infection, the *stx*-LB is located in the bacterial chromosome using site-specific recombination. All its expression is under the late RecA phage promoter. In the lysogenic state, the expression of prophage genes is repressed by the cI repressor. When the bacteria's SOS response is activated by DNA damage, or other stressors situations are encountered, the repressor cI is fragmented and the phage enters a lytic cycle. Then, during *stx*-LB induction by RecA, more particles of prophage are assembled and new Stxs are lately transcribed. Therefore, Stx increases, and its release into the environment occurs after host cell lysis (Wagner et al. 2001).

Certain experimental information suggest that the severity of the disease is correlated to the amount of Stx produced in the gut during infection. In this regard, Nawrocki et al. characterized two interactive mechanisms that increase Stx production and explained how the microbial communities could affect this expression: (1) Direct interactions between phage-infection susceptible *E. coli* and *stx*-converting bacteriophage from STEC can expand the Stx-producing population. The general abundance of bacteria in GM would suggest that interactions between *E. coli* and STEC strains have a high probability of occurrence. (2) Indirect amplification by secreted molecules, such as colicins and microcins, which promote phage induction (Nawrocki et al. 2020). These types of molecules are produced with the aim of killing competing strains. If the target cell is the STEC strain, the damage caused by the bacteriocins will activate the SOS response with the consequent induction of phages, production of Stx, and cell lysis. Phylogroup B2 includes strains that are in considerable amounts in the GM environment. In addition, they are strong bacteriocin-producing strains and form part of a protective niche against transient strains together with the rest of the resident strains.

De Sablet et al. said that the normal GM and the microbiota-secreted molecules are the major components of the digestive environment that could modulate Stx2 production (de Sablet et al. 2009). These authors investigated the influence of prokaryotic molecules produced by GM on Stx2 synthesis by EHEC O157. In their study, they demonstrated that one extracellular molecule produced by the *B. thetaiotaomicron strains* represses stx_2-mRNA expression and consequently Stx2 synthesis by inhibiting SOS and lytic cycle phage activation. On the other hand, Cordonnier et al. described that vitamin B12 stimulates EHEC to produce Stx2, and *B. thetaiotaomicron* has the capacity to inhibit toxin synthesis through environment depletion of vitamin B12 (Cordonnier et al. 2016). Therefore, people having some dysbiosis could present with a higher susceptibility to STEC infection and risk of developing severe disease. In addition, this conjecture could explain why STEC diseases occur more frequently in children (immature GM) than in adults. Other research focused on studying whether the expression of STEC genes involved in metabolism, colonization, and virulence was modulated in response to direct contact with GM. In the initial stages of gut colonization, other major virulence factors expressed by EHEC are those encoded at the LEE pathogenicity island: the T3SS, intimin (*eae*), and the translocated intimin receptor (*tir*), among others. Pacheco et al. described how EHEC uses available fucose by the microbiota to modulate EHEC pathogenicity and metabolism during the colonization stage. In fact, *E. coli* O157 regulate the

timing of virulence and metabolic gene expression in response to fucose, through the FusKR system to optimize its growth (Pacheco et al. 2012). Thus, colonization of mice by *B. thetaiotaomicron* increased the virulence of *C. rodentium*, worsening host pathology including crypt destruction and tissue edema (Curtis et al. 2014).

In contrast, when the pathogen is near to epithelial cells, two histidine sensor kinases/regulator systems (QseCB and QseEF) are activated by host adrenergic signals and by the microbiota-generated autoinducer AI-3 (quorum sensing), which finally leads to the expression of LEE virulence genes. In addition to this regulator response, QseCBEF also represses FusKR. This repression promotes virulence since the presence of both *fusK* and *fusR* represses LEE gene expression. The investigators speculate that FusKR repression of LEE expression in the mucus layer prevents EHEC strains to spend energy expressing virulence in a location where virulence factors are not necessary. Another conclusion has to do with the competition for nutrients. It is counterproductive for EHEC to utilize fucose in the intestinal lumen, when EHEC can efficiently use other carbon sources such as galactose, hexuronates, and mannose, which are not used by commensal *E. coli*. Additionally, EHEC is found to be associated with the intestinal epithelium, where it can utilize nutrients exclusively available at the surface of the epithelial cells, in contrast to commensal *E. coli* (Pacheco et al. 2012).

In this regard, Iversen H et al. investigated the global gene expression profile of the highly virulent strain EHEC O103:H25 in co-culture or by growing in the presence of a *B. thetaiotaomicron* conditioned medium (Iversen et al. 2015). They observed that (1) in the presence of metabolites found in the *B. thetaiotaomicron* conditioned medium, the genes involved in flagellar rotation of EHEC strain were transcriptionally upregulated causing an increase in tumbling activity (increased clockwise bias), which resulted in the inhibition of the motility of the strain. (2) Genes encoded in the LEE pathogenicity island such as T3SS, the EHEC-protease EspA, and factors involved in adherence to host cells were upregulated in direct contact with *B. thetaiotaomicron*. (3) Expression of *stx* phage genes, including those encoding Stx, were downregulated in a co-culture/conditioned medium (de Sablet et al. 2009). It is important to note that in contrast to commensal *E. coli* strains, which are found in the mucus layer, EHEC are located in close contact with the epithelium. Therefore, all these results suggest that this member of the microbiota could signal the adequate time for the pathogen colonization step. Thus, although the GM is considered to promote resistance to pathogen colonization, in some situations, *Bacteroides* can lead to an increase in the pathogen virulence.

Among the beneficial properties of GM, the effects of the SCFA (acetate, propionate, and butyrate), produced by GM through fermentation of no digestible carbohydrates, have been investigated (Ducarmon et al. 2019). SCFA can impair bacterial growth by affecting intracellular pH and metabolism. At lower pH, SCFAs are more prevalent in their non-ionized forms, and these non-ionized acids can diffuse across the bacterial membrane into the cytoplasm. Within the cytoplasm, they dissociate, leading to a lower intracellular pH. It was described that, in the presence of acetate, the metabolism of *E. coli* could be affected by preventing the biosynthesis of methionine, leading to the accumulation of toxic metabolites within the bacteria. It has

been observed that these protective effects depend on the concentrations of SCFAs, which are inversely related to the pH in different regions of the intestinal tract. Fukuda et al. showed that some *Bifidobacteria* contain a specific carbohydrate transporter (Fukuda et al. 2011). This strategy would help *Bifidobacteria* to accept fructose and produce acetate in the colon where glucose is scarce and, in that way, protect this intestinal area against STEC infections.

12.1.4.2 Gut Microbiota in STEC-Infected Patients

The pathogenesis of STEC infections is not completely understood and it seems that, besides the virulence potential of the infecting strains, other factors participate in the progression of the clinical symptoms. In addition to the virulence potential of this pathogen, there are different determinants of the host organism: age, diet, and genetic predisposition, which together with the human GM could influence the ability of STEC to efficiently colonize the gastrointestinal tract and would favor or not the progression to severe disease.

Tap et al. showed that the human gut microbiota is governed by the presence of *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria* and, in some cases, *Verrucomicrobia* bacterial phyla. Members of these taxa, including *Faecalibacterium*, *Ruminococcus*, *Eubacterium*, *Dorea*, *Bacteroides*, *Alistipes*, and *Bifidobacterium* genera, constitute a phylogenetic core shared among individuals (Tap et al. 2009). It has been shown that species belonging to *Bifid bacterium* and butyrate-producing bacteria, belonging to the Clostridiales order, might exert a variety of beneficial health effects (O'Callaghan and van Sinderen 2016; Rivière et al. 2016). Hence, a decrease in the relative abundances of *Bifidobacterium* species in the human colon has been associated with many disorders, such as inflammatory bowel disease, Crohn's disease and ulcerative colitis, irritable bowel syndrome, colorectal cancer, and increased gut permeability (O'Callaghan and van Sinderen 2016). The mentioned disorders lead to a general change in the gut microbiota composition, also favoring the colonization and proliferation of pathogenic microorganisms (de Vos and de Vos 2012).

Developments in molecular microbiology techniques, sequencing platforms, and bioinformatics during the past decade have allowed us to expand our knowledge on microbiota composition, dynamics, and impact on human health and disease. The use of shotgun metagenomic sequencing and different bioinformatic approaches, based on mapping of the reads onto databases and on the reconstruction of putative draft genomes, allows to investigate changes in the composition of the intestinal microbiota in samples from patients with EHEC/STEC infections, compared to healthy controls and children with HUS.

In Chilean children with DEC (including STEC)-causing diarrhea, changes in GM composition are characterized by an increase in *Proteobacteria* and *Bacteroidetes*, and a decrease in *Firmicutes* (Gallardo et al. 2017). These authors speculated that the increase in *Proteobacteria* can be partially explained by an increase in *Escherichia*/*Shigella* species as the cause of diarrhea, but also by other

members of *Enterobacteriaceae*, such as *Citrobacter* and *Enterobacter* strains. A subsequent study by the same group demonstrated that changes in GM during DEC infections were associated with changes in the intestinal metabolome (Gallardo et al. 2020). The integration of information from the microbiota and metabolome shows a strong correlation between a GM species and certain metabolites, such as histamine and L-ornithine, and deserves further investigation.

During diarrheal episodes in Israeli patients, Braun et al. also showed changes in phylum composition, with an increase in the abundance of *Proteobacteria* and *Bacteroidetes* and a decrease in *Firmicutes* (Braun et al. 2017). Similarly, in stool samples collected from STEC-infected patients from a nursery home in the province of Rome, Italy, Gigliucci et al. (2018) showed a lower abundance of the members of Bifidobacteriales and Clostridiales orders in comparison to control patients where those microorganisms predominated (Gigliucci et al. 2018). Jure et al. compared the GM composition of Argentinean children who developed HUS versus those who had only diarrhea after STEC infection and with healthy control patients (Jure et al. 2021). In this study, the authors used molecular techniques such as the 16S-seq to determine the microbial composition and relative abundance of specific microbiota in the examined sample (Logares et al. 2014). Considering that richness (number of microorganisms) and its biodiversity (number of species) are the major health indicators of GM, these parameters were evaluated by alpha indexes, such as Shannon's (which reflects the heterogeneity of a community based on the number of species present and their relative abundance), and the Chao's indexes (which reflects abundance and representation of each species in all samples) (del Campo-Moreno et al. 2018). To estimate the relationship between the different samples, weighted and unweighted UniFrac was used as a measure of β-diversity (Lozupone and Knight 2005). Similarities and differences between populations can also be noted when looking at the taxonomic abundance at the phylum level. Preliminary results obtained with these tools showed that the stool microbial composition of all cohorts (diarrhea, HUS, and healthy controls) was dominated by the three main phyla: *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, suggesting that intestinal destruction is not required for pathogen infection. In addition, the statistical analysis of β-diversity showed: (1) a lower abundance of *E. coli*/*Shigella* in samples of healthy controls; (2) differences in microbiome composition with a higher proportion of the following genus: *Bifidobacterium*, *Erysipelatoclostridium*, *Romboutsia*, *Dorea*, *Lactococcus*, and *Dysgonomonas*, in healthy controls and diarrhea cases compared to HUS patients; and (3) a highest statistical significance of *Bifidobacterium* in diarrhea compared to HUS cases. These differences in the composition seem to be related with STEC infections that do not progress to HUS (Jure et al. 2021). Further studies, including a larger number of fecal samples from healthy individuals, would be necessary to confirm the relationship between GM composition and progression to severe disease after STEC infections.

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