Gut Microbiota Characteristics in Ulcerative Colitis and other Gastrointestinal Diseases

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Gut microbiota refers to the collective assembly of micro-organisms in a given host environment. It includes, in addition to the well characterized bacterial communities, different life forms of viruses and fungi. Knowledge of these life forms and their functions is currently expanding paralleling with advances in microbiological next generation methodologies which enabled to recognize even unculturable organisms. In the normal health state, these life forms are in a symbiotic relationship with the host where they can provide some biological functions not executed by the host. Vitamin K biosynthesis is a well-known example of this. On the other hand, in disease state, these microbiota communities become disturbed where beneficial members are lost in favour of some pathological forms which may increase the pathological process. In this regard, the gastrointestinal tract is the best model to study these interactions given the direct contact of these microbiota communities with the host mucosal surfaces. In this review, we would revise some of these microbiota changes in different gastrointestinal diseases.

Gut microbiota changes in different gastrointestinal diseases provides an insight into the possible role of gut microbiota in the development of different GI diseases. However, the main question is "are these changes a cause or a result of a disease process". Moreover, trying to alter gut microbiota with a therapeutic intent is the best way to prove such a causal relationship where improvement of the disease process, after gut microbiota modulation, is a strong indicator of gut microbiota role in the pathogenesis of this disease.

INTRODUCTION

The term microbiota refers to the collective assembly of different microorganisms from different kingdoms (Prokaryotes, Eukaryotes) with their niche of activities including their cellular components, genetic materials, metabolites all embedded in their host environment **[1]**. It has been proposed, recently, that the human being is a holobiont encompassing own human cells and microbiota members with their different metabolic functions which intersect with the human metabolic and immunological functions **[2]**.

Traditionally, the gut microbiota numbers have been estimated at 10^{14} cells compared to own human cells of 10^{12} **[3].** Due to the huge numbers of microbiota inhabiting our body, these microbiota niches have been referred to our second genome **[4]**.

Under normal circumstances, gut microbiota exerts many physiological functions. This involves augmenting the host metabolism through the expression of many metabolism-related genes lacking in the human genome **[5]**. In this regard, most of the energy consumed by the colonic epithelial cells is provided by the gut microbiota as short chain fatty acids (SCFA) through digestion of unabsorbed carbohydrate **[6]**. Moreover, gut microbiota has a recognized role in the immune system regulation and programming **[7, 8]**. A traditional role that the gut microbiota

has long been known for is the protection against invasion and colonization by different pathogens **[9]**. One interesting recent application of this property is the production of the antibacterial microcins to bind siderophores produced by some pathogens and target these pathogens via siderophore receptor uptake acting exactly as Trojan horses **[10]**.

Although the gut microbiota shows daily variation due to many environmental effectors including diet, medications, smoking etc [11], it is a resilient community which can maintain its homeostasis continuously to maintain a symbiotic effect with the host environment **[12]**.

However, in disease states, particularly when prolonged, a state of dysbiosis ensues which seems to exceed the mere association to have detrimental effect on the host and disease progress **[13, 14]**.

Of particular interest is the gut microbiota changes in different gastrointestinal pathologies given the direct contact of the microbiota communities in the gut with the GI mucosa and related immune system. In this article, we will review gut microbiota disturbance patterns encountered in some of the most common GI diseases.

Gut Microbiota in Clostridium difficile infection (CDI)

A healthy gut microbiota is known to confer resistance against C. difficile infection. This fact has been derived from gut microbiota pattern changes observed in patients with CDI and the fecal microbiota transplantation (FMT) trials in patients suffering from C. difficile associated diarrhea with an estimated resolution rate approaching 95% in refractory and recurrent cases **[15]**.

Moreover, C. difficile infection is known to follow treatment with several classes of antibiotics, severely disturbs the gut microbiota facilitating invasion and colonization with CDI **[16, 17]**. Other predisposing factors to C. difficile infection such as proton pump inhibitors have profound effects on gut microbiome composition which may explain their association with C. difficile **[18]**.

In a nested case-control study, lower microbiota diversity and reduction of the *Clostridiales Incertae Sedis XI* were identified as a risk factor for being infected with C. difficile in hospitalized patients on antibiotics **[19]**. This shows the interaction between gut microbiota and other risk factors towards the development of C. difficile infection.

Moreover, the differences in gut microbiota which might predispose to CDI have been elucidated in a number of studies. For example, **Sangester et al**. demonstrated that patients with CDI infection had higher relative abundances of pathobionts including *Enterobacteriaceae, Peptostreptococcaceae* & the fungal genus *penicillium* compared to diarrheal non-CDI controls who were enriched in *Clostridiales and Bacteroidales*. However, this study failed to demonstrate any significant differences between the 2 study groups regarding the diversity or the abundance of butyrate producing bacteria **[20]**.

Another study that compared gut microbiota in patients with C. difficile infection (39 patients) and C. difficile negative nosocomial diarrhea (36 patients), found that C. difficile infection was associated with significantly lower diversity and richness of gut microbiota communities compared to healthy controls. This finding was more obvious in recurrent CDI cases. Significant decrease of SCFA producing anaerobes along with a significant increase of the gram negative *Proteobacteria* was observed with C. difficile infection **[21]**. The previous results were almost reproducible in another study where patients with C. difficile infection had lower gut microbiota diversity, richness, enrichment with *Proteobacteria & Fusobacteria* along with depletion of butyrate producing species. Interesting, similar features were observed with asymptomatic C. difficile carriers suggesting that gut dysbiosis is important for C. difficile presence **[22]**. A progressive decrease of the pathogenic organisms *Escherichia/Shigella* accompanied by a progressive increase of Bacteroides were observed from patients with CDI to carriers to healthy controls indicating a progressive increase of dysbiosis with disease. Similarly, more reduction in gut microbiota diversity has been shown associated with recurrent CDI **[23].**

Interestingly, gut microbiota disturbance during CDI could be predictive of recurrence where lower Bacteroidetes levels in CDI before antibiotic treatment was associated with an increased risk of recurrence while a pre-treatment high abundance of *Veillonella dispar* predicted against recurrence **[24]**.

A bidirectional relationship rather than a mere causal relationship best describes the interaction between gut microbiota and C. difficile, where gut microbiota perturbation predisposes to C. difficile infection and in turn C. difficile infection modulates the gut microbiota composition. Consistent with that, **Darkoh et al.** demonstrated that C. difficile was able to non-specifically induce indole producing bacteria to secrete excessive amounts of the antimicrobial indole which suppresses the growth of beneficial anaerobes in favour of pathogenic spp. This leads to a state of continuous dysbiosis which facilitates the recurrence of CDI after antibiotic treatment **[25]**.

Gut microbiota in IBD

Although the 2 phenotypes of IBD, namely, Crohn's disease (CD) and Ulcerative colitis (UC) share many pathogenic features including genetic, immunologic, and even epidemiologic features, the role of gut microbiota in the pathogenesis of both diseases seem to be different in terms of diversity, microbiota compositions and functional pathways as would be evident below. In this regard, it has been frequently shown that gut dysbiosis is deeper and more evident in CD compared to UC. This can have both diagnostic and therapeutic implications.

Gut microbiota in ulcerative colitis

Both experimental and human studies demonstrate gut microbiota disturbances. In human studies, "Diversion colitis" might be one early indication of the importance of gut microbiome in maintaining a healthy gut homeostasis and that its absence or disturbance might be responsible for colitis **[26]**. Also, the historical use of probiotics for treatment of UC, albeit with no significant effect, was another indication of the role gut microbiota perturbation in the pathogenesis of UC **[27]**.

A consistent depletion of the beneficial short chain fatty acid (SCFA) producing species as Fecalibacterium Prausnitzii in favour of the potentially pathogenic Enterobacteriaceae family (Escherichia/ Shigella) has been demonstrated in patients with UC **[28, 29]**. These pathogenic bacterial members have the ability to induce a proinflammatory responses enhancing the development and progression of UC **[30]**. This might be mediated through molecular mimicry or bacterial translocation with consequent activation of immune system **[31]**.

Gut microbiota can explain the effect of some factors predisposing to IBD pathogenesis and flares. An example of this would be the effect of dietary iron which leads to an increase of some iron-dependent species including the pathogens *E. coli and Klebsiella* which in turn can promote an inflammatory sequalae with IBD flare **[32]**.

It has been reported that patients with UC have gut dysbiosis manifested as a significant decrease of the health-associated species as *Roseburia and Akkermansia muciniphila* **[33]**, along with an increase of potential pathogens as *Fusobacterium, Helicobacter, E. coli, C. difficile* **[34-36]**. On the other hand, *Fecalibacterium Prausnitzii* seems to have a protective role against colitis as shown in the cohort of 116 patients with UC, where patients and their relatives had lower abundances of *Fecalibacterium Prausnitzii* compared to healthy controls. Moreover, *F. Prausnitzii* decrease was associated with higher risk of relapse and frequent relapses during the first year after induction of remission **[37]**.

Similarly, the protective role of *Bifidobacterium* against UC has been shown in a small Italian cohort of UC patients who showed significant decrease of *Bifidobacterium bifidum* species *(Bifidobacterium genus*) in active disease compared to both healthy controls and those in remission **[38]**.

Interestingly, infants born to mothers with IBD were shown to have similar gut dysbiosis with an increase of *Gammaproteobacteria* class and significant decrease of *Bifidobacterium* genus which could be ascribed to maternal IBD (after adjustment for other factors) **[39]**. This finding might indicate a role of gut microbiota in the familial aggregation of IBD cases. However, further long-term studies are needed to confirm or negate this assumption.

In a cohort of 228 subjects (including 75 UC patients) from the prospective PRISM and OSCCAR studies, patients with UC had lower abundances of the short chain fatty acids (SCFA) producers *Roseburia, Phascolarctobacterium & Leuconostocaceae*. In addition, patients with pancolitis had depletion of Odoribacter **[40]**. Other gut microbiota changes (including reduction of *Anaerostipes, Collinsella, Butyricicoccus Subdoligranulum, Dorea and*

enrichment of enterococcus), observed in this study were ascribed to age, medications, smoking and sample site. Hence, apart from disease activity & extent, many factors might take part in the dysbiosis observed in patients with IBD (UC & CD) with different effect sizes. These can include age, treatment status (including antibiotics and immunosuppressants) and external factors as smoking. This was confirmed in a recent multi-centre longitudinal study on IBD patients (303 CD patients, 228 UC patients and 161 controls), where geographical location, diet, history of resection surgery & alcohol consumption accounted significantly and to a variable extent, to the gut microbiota differences from healthy controls **[41]**.

A recent study on a Polish cohort of moderate to severe UC patients showed that those patients had higher abundances of *Actinobacteria & Proteobacteria* along with lower abundances of *Bacteroidetes and Verrucomicrobia* compared to healthy controls **[42]**.

In a cross-sectional study of an Italian cohort with IBD, IBD patients had higher Firmicutes, Proteobacteria, Verrucomicrobia & Fusobacteria and lower Fusobacteria and Cyanobacteria phyla compared to healthy controls. UC patients had, in addition, significant increase of actinobacteria compared to controls. At a lower phylogeny level, patients with CD had significant reduction of *Bacteroides, Fecalibacterium Prausnitzii, Prevotella, flavobacterium & Oscillospira.* Moreover, these patients had increased *Escherichia, Veillonella, streptococcus & Ruminococcus.* Although patients with UC had similar dysbiosis, they had opposite shift of F. Prausnitzii which was higher than controls but did not reach significance **[43].**

In addition to the dysbiosis frequently observed in patients with UC, the physiological interactions between gut microbiota and gene expression were shown to be significantly disturbed in UC with lower number of detected correlations between mucosal transcriptional profiles and gut microbiota in UC patients and their unaffected discordant twins compared to healthy controls. Transcriptional profile related to oxidative stress was increased in UC **[44]**.

While primary sclerosing cholangitis (PSC) is strongly related to UC **[45]** and although they share common features in the gut microbiota patterns, PSC was shown to be associated with an increase of some bacterial species independent of UC such as *Rothia, streptococcus, Enterococcus, Clostridium, Veillonella and Hemophilus*. On the other hand, some species were more abundant in UC compared to PSC-IBD as *Fusobacteriaceae* **[33]**.

Another cross-sectional study involving patients with PSC (with or without UC) and UC-alone patients showed similar results. Although all patient groups had a decrease in microbiota diversity compared to healthy controls, patients with PSC had unique microbiota profile which allowed their differentiation from both healthy controls and patients with UC with no hepatic disease. PSC patients had significantly higher abundance of *Veillonella*. Interestingly, the presence of associated UC had no significant effect on the gut microbiota pattern observed in PSC patients **[46]**.

Furthermore, experimental studies could also provide an insight into the role of gut microbiota in the development of UC. Germ free mice (GF) were shown to not develop colitis even with genetic modifications known to induce spontaneous colitis such as IL-10 deficiency **[47, 48]**.

Moreover, monocolonization of IL-2 deficient mice (mouse model of colitis) with *E. coli* was shown to promote colitis whereas monocolonization by *Bacteroides vulgatus* protected against the disease, both actions were shown to through an effect on lamina propria dendritic cell functions leading to either a proinflammatory or immunomodulatory actions respectively **[49]**.

In addition, *Fusobacterium* (isolated form UC patients) culture supernatant generated a condition similar to ulcerative colitis, with crypt abscesses & ulcers, when injected as enemas to mouse models **[50]**.

Gut Microbiota in Crohn's disease

Gut microbiota in CD varies between patients according to disease distribution as demonstrated by Willing et al., who showed that patients with Ileal Crohn's disease (ICD) had the lowest detected number of core microbiota members compared with Colonic CD (CCD) patients, in addition to opposite shifts in some microbiota members. ICD patients had the most significant changes with higher *fusobacterium, shigella, anaerovorax, acidaminococcus, citrobacter, Enterobacteriaceae & Veillonella* along with lower *Roseburia, Fecalibacterium,*

Ruminococcaeae incerta Sedis & unclassified spp. of lachnospiraceae compared to healthy controls. A predictive model of gut microbiota members identified patients with ICD with 100% accuracy compared to 91% in CCD patients **[51]**. However, the cohort of CD patients in this study was heterogenous including patients with active disease and others in remission.

A decrease of beneficial bacterial species in CD may be associated with diseases severity and recurrence. Again, *Fecalibacterium Prausnitzii* decrease in the mucosal samples from patients with CD at the time of surgery was associated with a higher risk of endoscopic recurrence 6 months after the surgery **[52]**.

In a prospective study on IBD patients (34 CD, 33 UC), patients with CD showed more disturbance of their gut microbiota relative to healthy controls over time and compared to UC patients. CD patients had significantly lower diversity, compared to healthy controls, and had a significant decrease of the microbiota members *Fecalibacterium, Oscillospira, Methanobrevibcater, collinsella, anaerostipes & Christensenellaceae*, at the time they demonstrated an increase in the potential pathogens fusobacterium and Escherichia compared to their controls. Interestingly, smoking in CD was associated with higher *Peptostreptococcaceae* compared to non-smokers who had higher abundances of *Eggerthella lenta*. Moreover, disease extent had some effect on gut microbiota composition. A discriminatory model was designed, based on the differential taxa, and could differentiate CD from UC and HC with an accuracy of 82% and 85% respectively **[53]**.

Moreover, **Clooney et al.** in their study, demonstrated that patients with CD had much lower diversity (more than UC patients) compared to healthy controls and were characterized by lower abundances of *Fecalibacterium Prausnitzii* and several *Eubacterium* species along with higher abundances of *Ruminococcus gnavus and Eggrethella lenta*. A discriminatory model, generated by Machine Learning, was created with the ability to discriminate CD from controls and UC from controls with an accuracy of 84% and 83% respectively. However, the accuracy of this model was dependent on the geographic location **[41]**.

Also, recently, **Sankarasubramanian et al.,** demonstrated that patients with CD had higher abundances of *C. ramosum, Ruminococcus lactaris, gnavus, clostridium clostridioforme, bolteae* compared to healthy controls who showed an association with *R. bromii, C. eutatus, C. catus and G. formicilis.* CD patients were found to be more dysbiotic compared to UC patients who had more overlap of their gut microbiota with healthy controls. Metabolomic analysis showed that CD patients had an increase of pathways associated with the pro-inflammatory benzoate degradation, amino acid & simple carbohydrate metabolism compared to UC patients **[54]**.

Gut microbiome in irritable bowel syndrome (IBS)

Irritable bowel syndrome is one of the most common digestive disorders world-wide with a global prevalence estimated at 9.2% based on Rome III criteria and 3.8% according to the Rome IV criteria as shown in a systematic review that involved 92 different adult populations **[55, 56]**. Some early studies have even estimated a probability of getting a diagnosis of IBS at 30% among those presenting with gastrointestinal symptoms at their GPs **[57]**. The underlying pathophysiology of IBS is not completely understood with many theories have been proposed involving many factors **[58]**.

The first indication of the role of gut microbiota in IBS was the condition termed post-infectious IBS which develops after acute gastroenteritis with associated gut microbiota alteration **[59]**. The gut microbiota composition and its contribution to the evolution of IBS has gained a lot of attention recently with the development of next generation sequencing and better understanding of the gut microbial environment.

In a cross-sectional study on IBS patients, the distribution of gut microbiota enterotypes was found to vary significantly according to IBS subtype, where the *Bacteroides* enterotype (lowest in *Methanobacteriales* abundance) was more common in IBS-diarrhoea predominant and IBS-mixed subgroups compared to healthy subjects who had the highest prevalence of *Prevotella* enterotype. Interestingly, Prevotella prevalence among the study groups had negative correlation with IBS severity. A microbial signature of 90 microbiota members, selected by machine learning, had the ability to differentiate

severe IBS from healthy controls and those with mild to moderate severity **[60]**.

Mucosa associated microbiota was shown to be disturbed as well in patients with IBS where in a population-based study from Sweden, the only findings related to an effect of IBS on gut microbiota was the reduced similarity, of mucosa associated microbiota MAM in patients with IBS compared to healthy controls. Otherwise, no significant differences could be detected between IBS patients and healthy controls in terms of diversity or microbiota composition **[61, 62]**.

A systematic review of 24 studies on adult and paediatric IBS patients, showed that patients with IBS had higher abundances of *Proteobacteria* phylum, *Enterobacteriaceae* & *lactobacillaceae* families and *Bacteroides* genus (of Bacteroidetes phylum) compared to healthy controls. Interestingly, higher abundances of *P. aeruginosa, Ruminococcus gnavus & C Difficile* were detected in IBS patients **[63]**.

Gut microbiota in celiac disease

Celiac disease is an immune disorder in genetically predisposed individuals where an adaptive immune response is mounted against gliadin fractions, released after ingestion of gluten, with subsequent damaging inflammatory response **[64]**.

Some gut microbiota members (*Bifidobacterium and Lactobacillus*) have been shown to digest gluten to non-immunogenic metabolites alleviating its pathogenic effect in genetically predisposed individuals **[65, 66]**. On the other hand, some pathogens (*Pseudomonas aeruginosa*) retrieved from the duodenum of patients with celiac disease has been shown to break down gluten into immunogenic substrates which activated gluten specific T-cells **[67]**.

The beneficial role of *Bifidobacterium and Lactobacillus* in patients with celiac disease might be ascribed to augmenting epithelial barrier function, reducing translocation of pathogenic bacterial species into the gut mucosa and modifying the immune response in the duodenum **[68]**. This is in addition to the previously mentioned role of these species in the favourable digestion of gluten **[65]**.

Assessment of the duodenal microbiota in children with celiac disease has shown significant decrease of the beneficial *Lactobacilli and Bifidobacteria* along with an increase of the pathogenic *Escherichia coli* compared to healthy controls **[69]**. Gut dysbiosis has been shown in patients with celiac disease who had lower abundances of *Fecalibacterium Prausnitzii, clostridium histolyticum, lituseburense and Bifidobacterium* along with higher *Prevotella & Bacteroides* compared to healthy controls. This was associated with impaired immunological response as shown by significantly lower IgA coating of both Prevotella and Bacteroides in those patients **[70]**.

Assessment of fecal microbiota in patients with celiac disease, consistently showed dysbiosis that coincided with that seen in duodenal mucosal biopsies where patients with celiac disease showed higher abundances of *staphylococcus, Clostridium leptum, E. coli and Bacteroides* compared to controls who had higher *Bifidobacterium*. Interestingly, a gluten free diet led to a partial improvement of these dysbiotic features where *staphylococcus* and E. coli returned to normal levels after treatment **[71]**. However, a gluten free diet for more than 2 years failed to completely restore the gut microbiota composition where still a treated cohort of children with celiac disease exhibited higher *Bacteroides, Shigella, Salmonella & Klebsiella* and lower *Bifidobacteria & lactobacillus* compared to healthy controls **[72]**. Interestingly this study identified some fecal metabolites as markers of celiac disease including ethylacetate, octylacetate, some SCFA and some amino acids.

Persistence of symptoms in patients with celiac disease despite a gluten free diet has been associated with alterations of duodenal microbiota with higher proteobacteria and lower *Bacteroidetes & Firmicutes* found in those symptomatic treated patients compared to healthy controls **[73]**.

Role of neglected microbiota members (Mycobiota and Viromes)

Recently, the role of other players of the gut microbiota in different GI diseases has gained attention. Those include both the fungal communities (Mycobiota) and the viral component (viromes).

In UC, differences have been spotted between patients and healthy controls regarding core virome members including specifically the Caudovirales which become decreased at the time *Enterobacteriaceae phages* increased **[74]**. Similarly, patients with CD have been shown to have differences in their virome community

compared to healthy controls. Remarkably, CD patients have shown differences in their virome content from different GI segments **[75]**. Also, an interaction between specific microbiota members and their lytic phages have been shown in CD [76]. Moreover, CD patients have been shown to have fungal dysbiosis with an increase of their fungal/bacterial ratios associated with an increase of their C*andida albicans* and lower *saccharomyces cerevisiae* **[77]**.

Still, these 2 aspects of the gut microbiota require further studies and assessment in both observational and interventional studies to elucidate their exact roles in the pathogenesis of different GI diseases.

Faecal Microbiota Transplantation (FMT); a possible treatment and a prove

Given the above-mentioned changes, gut microbiota modulation has developed as an appealing target for treating these disease processes with gastrointestinal diseases representing the best field to examine such a treatment modality given the direct contact of the gut microbiota with diseases gut mucosa.

These therapeutic modalities included the use of probiotics, antibiotics, and the rapidly expanding field of fecal microbiota transplantation which showed promising results in IBD [78-85]. Also, FMT has shown a long success story in C. difficile infection which enabled complete cure with rare recurrence after single treatment **[15]**. However, lack of standardization of the process is a major shortcoming before approval of FMT for different GI diseases.

Conclusions and future perspectives

The above-mentioned gut microbiota changes in different gastrointestinal diseases provides an insight into the possible role of gut microbiota in the development of different GI diseases. However, the main dilemma of a causal relationship persists with the main question is "are these changes a cause or a result of a disease process". This can be answered by conducting well designed longitudinal studies that explore the gut microbiota changes over time before disease onset in at-risk individuals and after disease onset both before treatment and after treatment. Moreover, trying to alter gut microbiota with a therapeutic intent is the best way to prove such a causal relationship where improvement of the disease process, after gut microbiota modulation, is a strong indicator of gut microbiota role in the pathogenesis of this disease.

Another area of interest which needs further studies is the role of gut Mycobiota and gut virobiota given the scarce literature exploring these microbiota members.

Abbreviations

UC: Ulcerative colitis

CD: Crohn's disease

IBD: Inflammatory bowel diseases

C. difficile: Clostridium difficile

FMT: Fecal Microbiota Transplantation

GI: Gastrointestinal

PSC: Primary sclerosing cholangitis

SCFA: Short chain fatty acids

Competing interests

The authors declare that they have no competing interests.

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Availability of data and material

All data collected, generated or analyzed during this study are included in this published article.

HIGHLIGHTS:

- Gut microbiota refers to the collective assembly of micro-organisms in a given host environment.
- It includes, in addition to the well characterized bacterial communities, different life forms of viruses and fungi.
- Gut microbiota changes in different gastrointestinal diseases provides an insight into the possible role of gut microbiota in the development of different GI diseases.
- Another area of interest which needs further studies is the role of gut Mycobiota and gut virobiota given the scarce literature exploring these microbiota members.

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