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Specificities of the intestinal microbiota in patients with inflammatory bowel disease and *Clostridium difficile* infection

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Short title: Microbiota in IBD with CDI

Abbreviations: inflammatory bowel diseases (IBD), healthy subjects (HS), Crohn's disease (CD), ulcerative colitis (UC)

Abstract

Background and Aims: *Clostridium difficile* infection (CDI) is a common complication in inflammatory bowel disease (IBD) and has been associated with poor IBD outcome. Intestinal microbiota composition in IBD patients with CDI has not been specifically evaluated to date.

Methods: The fecal microbiota of 56 IBD patients, including 8 in flare with concomitant CDI, 24 in flare without CDI, and 24 in remission, as well as 24 healthy subjects, was studied using 16S sequencing. Analysis was performed using the Qiime pipeline.

Results: Compared to IBD patients without CDI, IBD patients with CDI had more pronounced dysbiosis with higher levels of *Ruminococcus gnavus* and *Enterococcus* operational taxonomic units (OTUs) and lower levels of *Blautia* and *Dorea* OTUs. Correlation network analysis suggested a disrupted ecosystem in IBD patients in flare, particularly in those with CDI.

Conclusions: In patients with IBD, CDI is associated with a more pronounced intestinal dysbiosis with specific alterations in intestinal microorganisms.

Keywords: IBD, *Clostridium difficile*

Introduction

Clostridium difficile infection (CDI) is a common complication of inflammatory bowel disease (IBD) and has been associated with a poor IBD outcome[1]. CDI typically occurs after a transient microbiota perturbation in the gut. Thus, antibiotic intake is a major risk factor in the general population. In IBD, as the gut microbiota is basally perturbed (i.e., dysbiosis)[2], CDI can occur even without an antibiotic trigger.

Microbiota composition in patients with CDI is imbalanced and characterized by decreased biodiversity[3,4], as well as notable increases and decreases in several taxa that are known to be increased and decreased in IBD[4], respectively. For example, *Bifidobacterium* and members of Clostridium clusters IV and XIVa (such as *Faecalibacterium prausnitzii* or *Roseburia intestinalis*) were decreased, while Enterobacteriaceae and *Enterococcus* were increased[5]. However, intestinal microbiota composition in IBD patients with CDI has not been specifically evaluated to date.

Results

Population, microbiota composition and diversity

Our study population was composed of 56 IBD patients, including 8 in flare with concomitant CDI, 24 in flare without CDI, and 24 patients in remission, as well as 24 healthy subjects (HS) (Table 1). IBD patients in flare without CDI and patients in remission were matched to patients in flare with CDI (3:1) based on IBD subtype and topography. None of the study participants reported having taken antibiotics or using colon-cleansing products for at least 2 months prior to enrollment.

Beta diversity analysis showed a clustering of samples by disease phenotype and a progressive microbiota shift from HS to IBD in remission, to IBD in flare and finally to IBD in flare with CDI (Figure 1A-B). Compared with the HS samples, the alpha diversity (assessed by four different indexes) was significantly decreased in IBD patients, particularly in samples from patients in flare (Figure 1C, Suppl Figure 1A-B). There was no significant difference between patients with and without CDI.

As expected, most of the bacteria from both the IBD and HS samples belonged to the phyla Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria (Figure 1D), and the distribution of these phyla in the patients was in agreement with published data[6]. Firmicutes and Bacteroidetes were the most abundant in HS. In IBD patients, particularly those in flare, there was a decrease in Firmicutes (mostly from the Ruminococcaceae family) associated with an increase of Proteobacteria (mostly from the Enterobacteriaceae family ; Figure 1D, Suppl Figure 1C).

Differential bacterial composition and altered microbial network in CDI

To identify more precisely the impact of CDI on IBD microbiota, we directly compared the bacterial composition of IBD patients in flare, both with and without CDI, using LEfSe (Linear discriminant analysis Effect Size)[7]. We identified several OTU that differentiated patients with and without CDI (Figure 1E, Suppl Table 1). Several *Blautia* and *Dorea* OTUs were decreased in patients with CDI, whereas several *Clostridium*, *Ruminococcus gnavus* and *Enterococcus* OTUs were increased in patients with CDI.

To evaluate globally the microbial gut ecosystem and its alterations in IBD and CDI, we built a microbial correlation network by phenotype (Figure 2). In HS, we observed a highly connected network between many bacterial OTUs, suggesting the presence of a balanced and cooperative ecosystem. Although smaller than that of HS, the correlation network was also well-connected in IBD patients in remission. In contrast, the correlation network was disrupted in IBD patients in flare, with separated bacterial clusters being detected. For IBD patients in flare who also had CDI, the alterations were even more pronounced with a lower relative connectedness (2.1 vs 5.4 in IBD patients in flare without CDI, $p<10^{-8}$) and no interaction between several groups of bacteria, suggesting a loss of cooperation in the ecosystem.

Discussion

To the best of our knowledge, this report presents the first detailed analysis of the intestinal microbiota of IBD patients both with and without CDI. In accordance with previous studies, we confirmed that the intestinal microbiota of IBD patients is dysbiotic. The current study showed that CDI in patients with IBD flare is associated with a more severe dysbiosis than in an IBD flare without CDI. This infection is characterized by specific alterations in intestinal microorganisms with an impaired microbial ecosystem. Interestingly, among the decreased bacterial OTUs in patients with CDI, we identified many genera known to be deficient in IBD compared to HS, such as *Blautia* and *Dorea*[6]. In addition, the *Ruminococcus gnavus* and *Enterococcus* OTUs that were increased in the patients with CDI have also been shown to be overrepresented in IBD patients compared to HS[8,9]. This finding indicates a deeper dysbiosis

in IBD patients with CDI. Moreover, several CDI features in non-IBD patients, such as increased numbers of *Enterococcus*[5], were also found in the IBD patients.

In conclusion, this study showed that in patients with IBD, CDI is associated with a more pronounced intestinal dysbiosis. Whether this feature is a cause or a consequence of CDI is not established. Therefore, the next step will be to investigate microbiota factors associated with the risk of developing CDI in IBD as a prerequisite for preventive intervention.

Materials and Methods

Patients and sample collection

All patients were recruited at the Gastroenterology Department of the Saint Antoine Hospital (Paris, France) and provided informed consent. Approval was obtained from the local ethics committee (Comite de Protection des Personnes Ile-de-France IV, Suivitheque study). A diagnosis of IBD was defined by clinical, radiological, endoscopic and histological criteria. A flare was determined by the physician in charge of the patient based on clinical and biological data. None of the study participants reported having taken antibiotics or using colon-cleansing products for at least 2 months prior to enrollment. Patient characteristics are presented in Table 1. Samples from healthy subjects (HS) and from IBD patients without a *Clostridium difficile* infection were described previously [2]. Whole stools were collected in sterile boxes and immediately homogenized, and 0.2 g aliquots were frozen at −80°C for further analysis.

Fecal DNA extraction

Genomic DNA was extracted from 200 mg of feces as previously described [1]. Following microbial lysis with both mechanical and chemical steps, nucleic acids were precipitated in isopropanol for 10 minutes at room temperature, incubated for 15 minutes on ice and centrifuged for 30 minutes at 15,000 *g* and 4°C. Pellets were suspended in 112 µL of phosphate buffer and 12 µL of potassium acetate. After RNase treatment and DNA precipitation, nucleic acids were recovered via centrifugation at 15,000 *g* and 4°C for 30 minutes. The DNA pellet was suspended in 100 µL of TE buffer.

16S rRNA gene sequencing

After extraction, total DNA concentration was measured using PicoGreen (Invitrogen), and global 16S gene DNA copy numbers were measured using a qPCR method adapted from Maeda *et al*. [10], enabling inhibition effect estimation and DNA concentration adjustment. The sequence region of the 16S rRNA gene spanning the variable region V3-V5 was amplified using the broad-range forward primer, For16S_519 (CAGCMGCCGCGGTAATAC), and reverse primer, Rev16S_926 (CCGTCAATTCMTTTGAGTTT). The amplification reaction (initial activation step at 94°C for 1 min, followed by 30 cycles of 94°C for 15 s, 43°C for 15 s, 68°C for 45 s and a final incubation step at 68°C for 1 min) was performed in a total volume of 100 µL containing 1X PCR buffer, 2 mM $MgSO_4$, 1 U of DNA High Fidelity Taq Polymerase (Invitrogen, Carlsbad, CA), 625 nM of each barcoded primer (IDT), 250 µM of each dNTP (Invitrogen) and the concentration-adjusted DNA sample. A bidirectional library was prepared using the One Touch2 Template Kit and sequenced on PGM Ion Torrent using the Ion PGM Sequencing 400 Kit (Life Technologies, Carlsbad, CA).

16S rRNA gene sequence analysis

The sequences were demultiplexed and quality filtered using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) software package[11]. The sequences were trimmed for barcodes and PCR primers and were binned for a minimal sequence length of 200 bp. The sequences were then assigned to (OTUs) using the UCLUST algorithm [12] with a 97% threshold pairwise identity and taxonomically classified using the Greengenes reference database[13]. Rarefaction was performed (10,000 sequences per sample) and used to compare OTU abundances across samples. Principal component analyses (PCA) of the Bray Curtis distance, with each sample colored according to disease phenotype, were built and used to assess the variation between experimental groups. The number of observed species, as well as the Shannon, Simpson and Chao1 diversity indexes, were calculated using rarefied data (depth = 10,000 sequences/sample) and used to characterize species diversity in a community.

Clostridium difficile testing

Diagnosis of CDI was performed using a stool culture on selective medium (TCCA, taurocholate cycloserine cefoxitine medium) and a stool cytotoxicity assay on MRC-5 cells. In cases in which a positive culture and negative stool cytotoxicity assay were obtained, a toxigenic culture (determination of the isolate's ability to produce toxins in vitro) was performed.

Statistical analysis

GraphPad Prism version 6.0 (San Diego, CA) was used for all analyses and graph preparation. For all graphical data, the results are expressed as the mean ± SEM, and statistical analyses were performed using the 2-tailed nonparametric Mann–Whitney *U*-test or the Kruskal-Wallis test with Dunn's multiple comparison test. Statistical significance of the sample grouping for

the beta diversity analysis was performed using the Permanova method (9999 permutations). Differences with a P value less than 0.05 were considered to be significant.

Comparisons between IBD patients with and without CDI was performed using the linear discriminant analysis (LDA) effect size (LEfSe) pipeline [7].

A correlation network was built using an OTU table and the Spearman correlation. Only OTUs present in >50% of the samples were used. The p values were corrected using the Benjamini and Hochberg procedure to control for the false discovery rate (FDR). Correlation network figures were built using Cytoscape 3.1.1. The relative connectedness of the networks was calculated by dividing the number of significant interactions (edges) by the number of taxa (nodes) in the network. Statistical significance was assessed by a chi-square test.

Authors' Contributions

HS designed and supervised the study. VL, SJ and CM provided technical help. HS, CL, JK, GL, AB, INL, JC, PS, LB, recruited the patients, HS performed the analysis and interpreted the data. HS wrote the manuscript.

The authors state that the manuscript, including related data, figures and tables, has not been previously published and that the manuscript is not under consideration elsewhere.

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Conflicts of interest

The authors disclose no conflicts of interest.

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Figure Legends

Figure 1: Bacterial microbiota biodiversity and composition. (A) Beta diversity. Principal coordinate analysis of the Bray Curtis distance with each sample colored by the disease phenotype. PC1, PC2, and PC3 represent the top three principal coordinates that captured the most diversity, with the fraction of diversity captured by that coordinate shown as a percentage. (B) PC1 value according disease phenotype (t test, ** = $p< 0.01$; *** = $p< 0.001$). (C) OTU number describing the diversity of the bacterial microbiota in the different groups studied (t test,* = $p< 0.05$; ** = $p< 0.01$; *** = $p< 0.001$). (D) Global composition of bacteria at the phyla level. HS and patient sub-groups are labeled on the X-axis and expressed as the relative OTU abundance per group. (E) Bacterial taxa differentially enriched in IBD patients in flare both with and without CDI (generated using LeFSE analysis, LDA score >2).

Figure 2: Bacterial microbiota correlation network. Correlation network for the HS, IBD in remission, and IBD in flare both without and with CDI was generated using Cytoscape. Each circle (node) represents an OTU, the color represents its bacterial phylum (blue: Firmicutes; green: Bacteroidetes; orange: Proteobacteria), and its size represents the number of direct edges that it has. The edge color indicates the magnitude of the distance correlation; green indicates a positive correlation and red indicates a negative correlation (determined using the Spearman test). Statistical significance was determined for all pairwise comparisons; only significant correlations (p value < 0.05 after FDR correction) are displayed.

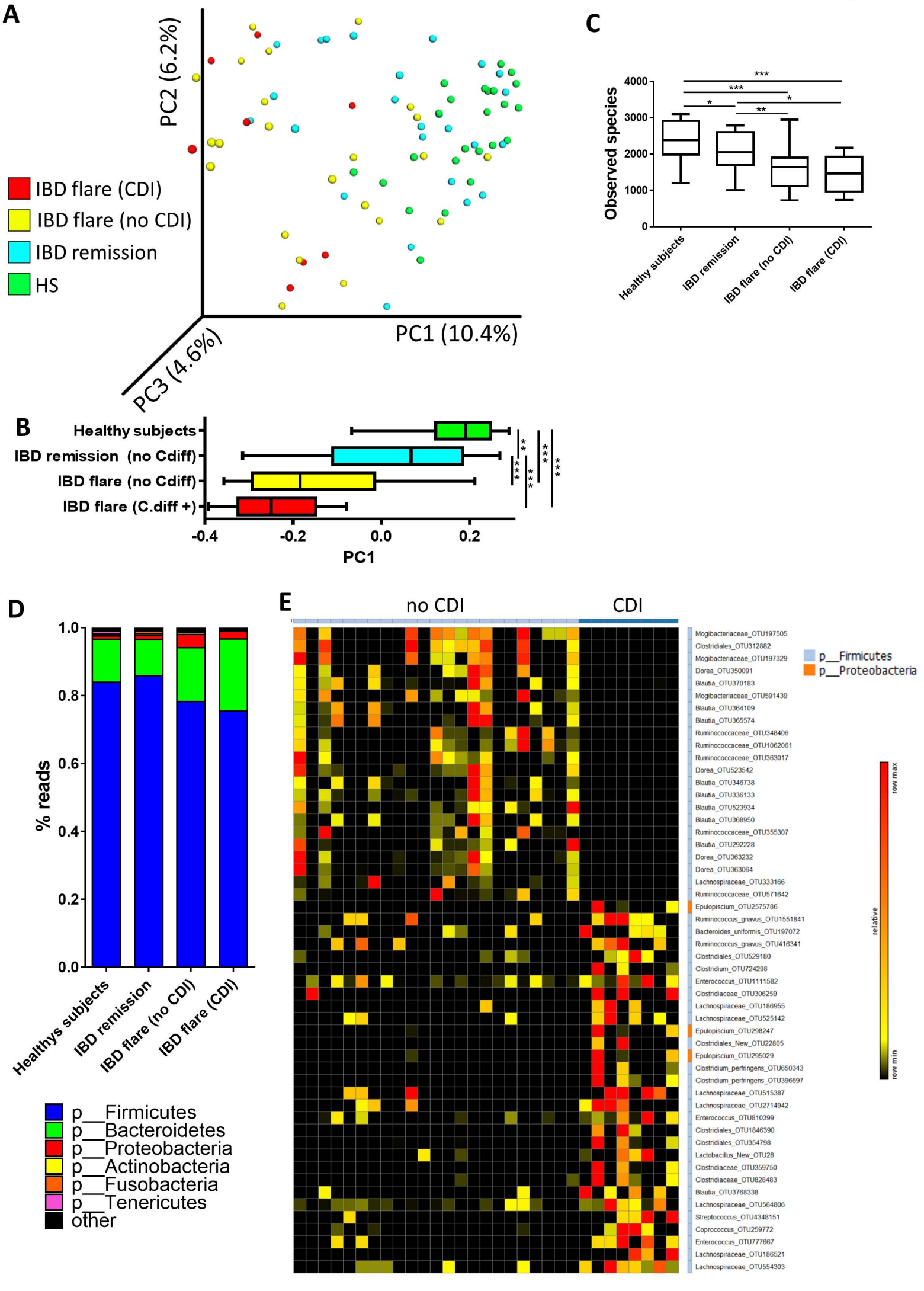
Supplementary Materials and Methods

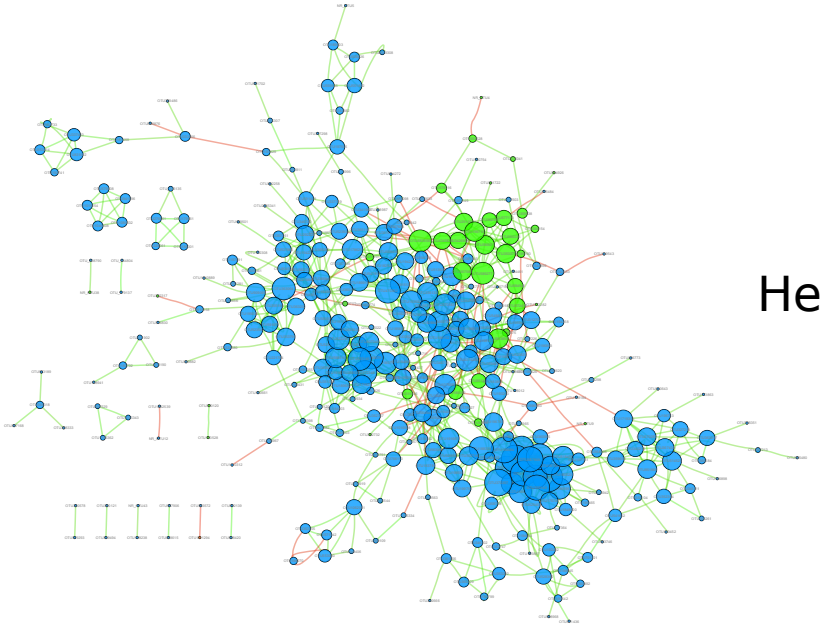
273

Supplementary Figure Legend

Supplementary Figure 1: Bacterial microbiota biodiversity and composition. (A) Chao1 diversity index of the bacterial microbiota in the different groups studied. (B) Shannon diversity index of the bacterial microbiota in the different groups studied (t test,* = p< 0.05; ** = p< 0.01; *** = p< 0.001). (C) Global composition of bacteria at the family level. HS and patient sub-groups are labeled on the X-axis and expressed as the relative OTU abundances per group.

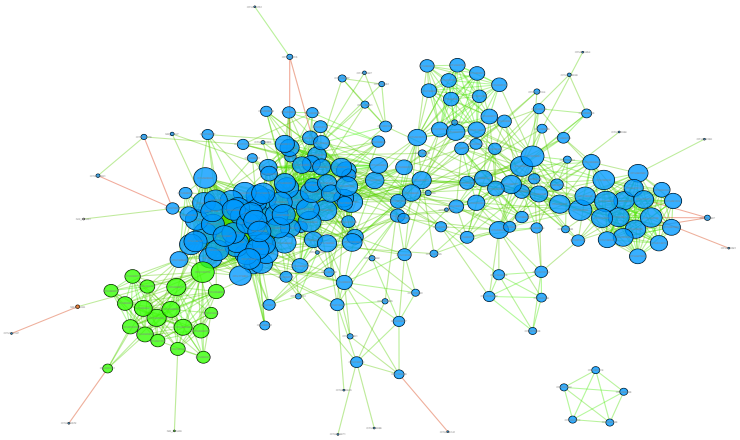
Figure 1

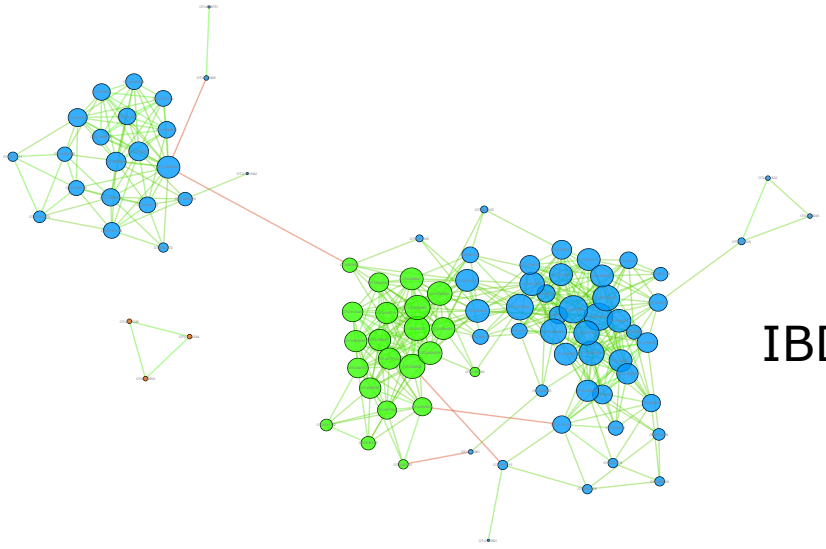

A
PC2 (6.2%)
PC1 (10.4%)
PC3 (4.6%)
IBD flare (CDI)
IBD flare (no CDI)
IBD remission
HS
C
Observed species
4000
3000
2000
1000
0
Healthy subjects
IBD remission
IBD flare (no CDI)
IBD flare (CDI)
B
Healthy subjects
IBD remission (no Cdiff)
IBD flare (no Cdiff)
IBD flare (C.diff +)
-0.4
-0.2
0.0
0.2
PC1
D
% reads
1.0
0.8
0.6
0.4
0.2
0.0
Healthys subjects
IBD remission
IBD flare (no CDI)
IBD flare (CDI)
p__Firmicutes
p__Bacteroidetes
p__Proteobacteria
p__Actinobacteria
p__Fusobacteria
p__Tenericutes
other
E
no CDI
CDI
Mogibacteriaceae_OTU197505
Clostridiales_OTU312882
Mogibacteriaceae_OTU197329
Dorea_OTU350091
Blautia_OTU370183
Mogibacteriaceae_OTU591439
Blautia_OTU364109
Blautia_OTU365574
Ruminococcaceae_OTU348406
Ruminococcaceae_OTU1062061
Ruminococcaceae_OTU363017
Dorea_OTU523542
Blautia_OTU346738
Blautia_OTU336133
Blautia_OTU523934
Blautia_OTU368950
Ruminococcaceae_OTU355307
Blautia_OTU292228
Dorea_OTU363232
Dorea_OTU363064
Lachnospiraceae_OTU333166
Ruminococcaceae_OTU571642
Epulopiscium_OTU2575786
Ruminococcus_gnavus_OTU1551841
Bacteroides_uniformis_OTU197072
Ruminococcus_gnavus_OTU416341
Clostridiales_OTU529180
Clostridium_OTU724298
Enterococcus_OTU1111582
Clostridiaceae_OTU306259
Lachnospiraceae_OTU186955
Lachnospiraceae_OTU525142
Epulopiscium_OTU298247
Clostridiales_New_OTU22805
Epulopiscium_OTU295029
Clostridium_perfringens_OTU650343
Clostridium_perfringens_OTU396697
Lachnospiraceae_OTU515387
Lachnospiraceae_OTU2714942
Enterococcus_OTU810399
Clostridiales_OTU1846390
Clostridiales_OTU354798
Lactobacillus_New_OTU28
Clostridiaceae_OTU359750
Clostridiaceae_OTU828483
Blautia_OTU3768338
Lachnospiraceae_OTU564806
Streptococcus_OTU4348151
Coprococcus_OTU259772
Enterococcus_OTU777667
Lachnospiraceae_OTU186521
Lachnospiraceae_OTU554303
p__Firmicutes
p__Proteobacteria
row max
relative
row min



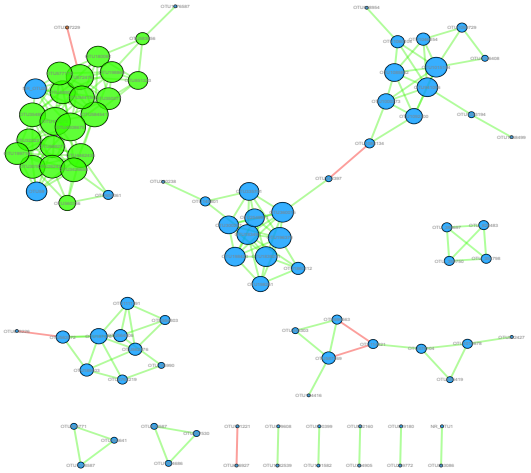
Healthy subjects

IBD remission



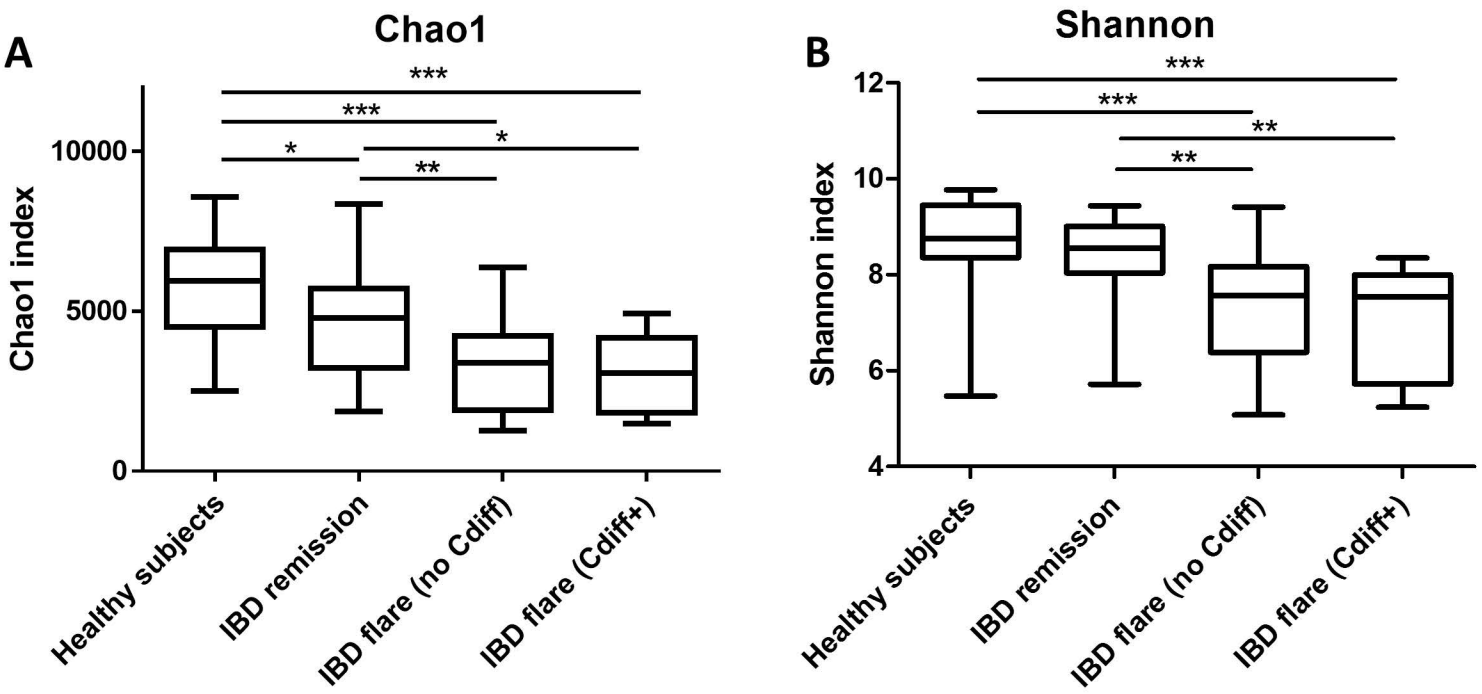


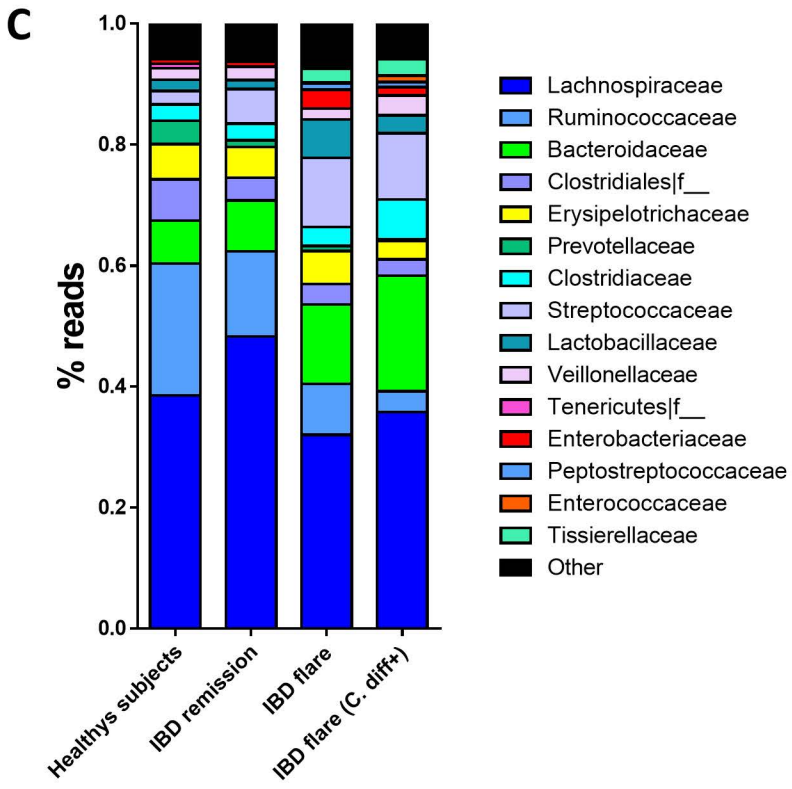
IBD flare (no CDI)



IBD flare (CDI)

Supplementary Figure 1





Supplementary Table 1 : Lefse output : IBD without CDI vs IBD with CDI

OTU	Increased in	LDA	pvalue
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus.s__New_ReferenceOTU12	no_ICD	2,709772134	0,042547194
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU368950	no_ICD	2,126381739	0,042417294
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU523934	no_ICD	2,298329146	0,042612179
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU292228	no_ICD	2,334277874	0,042547194
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU365574	no_ICD	2,395775029	0,042547194
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU364109	no_ICD	2,464763391	0,042514711
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU346738	no_ICD	2,478248177	0,04264468
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU336133	no_ICD	2,652839773	0,013516916
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU370183	no_ICD	3,497785175	0,006901304
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales_OTU312882	no_ICD	2,138634014	0,029440095
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Coprococcus.s__OTU509416	no_ICD	2,764876219	0,009538398
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea.s__OTU350091	no_ICD	2,222337607	0,042514711
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea.s__OTU523542	no_ICD	2,354482673	0,029463885
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea.s__OTU363232	no_ICD	2,506881192	0,029535294
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea.s__OTU363064	no_ICD	3,048426578	0,034512032
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae_OTU333166	no_ICD	2,568375203	0,042547194
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Mogibacteriaceae.g__.s__OTU197505	no_ICD	2,197417666	0,01343149
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Mogibacteriaceae.g__.s__OTU197329	no_ICD	2,229470457	0,042612179
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Mogibacteriaceae.g__.s__OTU591439	no_ICD	2,312112231	0,029630589
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Peptostreptococcaceae.g__.s__OTU547714	no_ICD	2,07927405	0,041764625
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__.s__OTU348406	no_ICD	2,351951641	0,0291788
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__.s__OTU330578	no_ICD	2,545676932	0,042482233
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__.s__OTU571642	no_ICD	2,548597635	0,042547194
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__.s__OTU355307	no_ICD	2,593950091	0,042514711
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__.s__OTU363017	no_ICD	2,896417034	0,042612179
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__.s__OTU561607	no_ICD	2,963892372	0,042612179
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__.s__OTU1062061	no_ICD	3,13030404	0,020237338
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__New_ReferenceOTU26	ICD	3,159333048	0,020836678
k__Bacteria.p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Bacteroidaceae.g__Bacteroides.s__uniformis_OTU197072	ICD	2,105371372	0,039902891
k__Bacteria.p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Barnesiellaceae.g__.s__OTU3090117	ICD	2,280992361	0,014787029

k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__OTU3768338	ICD	2,035548356	0,007239643
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia.s__producta_OTU326865	ICD	2,289243933	0,033614828
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Blautia_362958	ICD	2,798873291	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__.s__306259	ICD	2,151239488	0,017782486
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__.s__OTU190003	ICD	2,153583951	0,024479083
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae_828483	ICD	3,047627085	0,005981652
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae_359750	ICD	3,44732167	0,000365284
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__.s__OTU606927	ICD	3,612199191	0,008833481
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__.g__.s__534957	ICD	2,017894414	0,022198769
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales_1846390	ICD	2,072160288	0,00240333
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales_New_CleanUp_ReferenceOTU22805	ICD	2,086165919	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__.g__.s__OTU198251	ICD	2,147435522	0,021612188
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__.g__.s__New_CleanUp_ReferenceOTU23027	ICD	2,176783737	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__.g__.s__OTU529180	ICD	2,25867102	0,027619597
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales_New_CleanUp_ReferenceOTU36592	ICD	2,344953413	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__.g__.s__OTU342397	ICD	2,865633643	0,0291519
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__.g__.s__354798	ICD	3,27040575	0,00597103
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales_OTU628226	ICD	3,654128818	0,002459037
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__Clostridium.s__perfringens_650343	ICD	2,262257751	0,00240333
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__Clostridium.s__724298	ICD	2,488412305	0,00240333
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__Clostridium.s__perfringens_396697	ICD	2,780555912	0,000365284
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__Clostridium.s__butyricum_OTU541328	ICD	3,091233772	0,044571347
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__Clostridium.s__4352349	ICD	3,582042637	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Clostridiaceae.g__Clostridium.s__2670715	ICD	4,231520538	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Coprococcus.s__OTU300297	ICD	2,39102753	0,041918675
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Coprococcus.s__OTU259772	ICD	2,868751036	0,013633486
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea.s__OTU196176	ICD	2,073798121	0,044719844
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea.s__New_CleanUp_ReferenceOTU33563	ICD	2,236455645	0,014787029
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Enterococcaceae_OTU225919	ICD	2,031815342	0,020070872
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Enterococcaceae.g__Enterococcus.s__OTU777667	ICD	2,231527429	0,001438634
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Enterococcaceae.g__Enterococcus.s__OTU810399	ICD	2,960440267	0,001510648
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Enterococcaceae.g__Enterococcus.s__OTU1111582	ICD	3,316570625	0,041876848
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Epulopiscium.s__1658639	ICD	2,619604976	0,014787029
k__Bacteria.p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales.f__Erysipelotrichaceae.g__.s__OTU262095	ICD	2,014896732	0,048335369

k__Bacteria.p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales.f__Erysipelotrichaceae.g__.s__OTU356760	ICD	2,248239999	0,01665132
k__Bacteria.p__Firmicutes.c__Erysipelotrichi.o__Erysipelotrichales.f__Erysipelotrichaceae.g__.s__OTU360238	ICD	2,365767148	0,019426341
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Pasteurellales.f__Pasteurellaceae.g__Haemophilus.s__parainfluenzae_OTU160015	ICD	2,055478535	0,014015076
k__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Pasteurellales.f__Pasteurellaceae.g__Haemophilus.s__parainfluenzae_OTU1016422	ICD	2,175725828	0,01836869
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__New_CleanUp_ReferenceOTU29281	ICD	2,014500266	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__New_CleanUp_ReferenceOTU1777	ICD	2,033502038	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__OTU186955	ICD	2,058689631	0,016187289
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae_OTU191999	ICD	2,10430887	0,013054349
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae_OTU2714942	ICD	2,112447997	0,020744402
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__OTU4426877	ICD	2,144346253	0,028308724
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae_OTU515387	ICD	2,222776486	0,020529372
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__OTU196990	ICD	2,278921856	0,012400722
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__OTU186521	ICD	2,285395169	0,00240333
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__OTU525142	ICD	2,307560565	0,022351007
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__OTU554303	ICD	2,393225748	0,000249348
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae_OTU196370	ICD	2,961263016	0,013515946
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae_New_CleanUp_ReferenceOTU41303	ICD	2,973537047	0,01460627
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__.s__OTU564806	ICD	3,059346739	0,009969624
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae_OTU198423	ICD	3,13442702	0,012613888
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus.s__zeae_679245	ICD	2,219688181	0,014635653
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus.s__794205	ICD	2,222146634	0,00240333
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus.s__692823	ICD	2,275755931	0,020107819
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus.s__OTU91147	ICD	2,372015581	0,016187289
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus.s__New_ReferenceOTU28	ICD	2,565255835	0,008021195
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus.s__manihotivorans_OTU518033	ICD	2,649528161	0,00240333
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Ruminococcus.s__gnavus_OTU2575651	ICD	2,057219895	0,033823367
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Ruminococcus.s__gnavus_OTU183495	ICD	2,122071431	0,043954718
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Ruminococcus.s__gnavus_OTU4338803	ICD	2,144541001	0,020486998
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Ruminococcus.s__gnavus_OTU1551841	ICD	2,153436752	0,020738111
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Ruminococcus.s__New_CleanUp_ReferenceOTU24360	ICD	2,233794941	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Ruminococcus.s__gnavus_OTU1839271	ICD	2,250891702	0,011241596
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Ruminococcus.s__gnavus_OTU416341	ICD	2,603989613	0,028308724
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Ruminococcus.s__gnavus_OTU360015	ICD	3,948864961	0,044232625
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Streptococcaceae.g__Streptococcus.s__4348151	ICD	2,011583756	0,002410416

k__Bacteria.p__Proteobacteria.c__Betaproteobacteria.o__Burkholderiales.f__Alcaligenaceae.g__Sutterella.s__OTU215097	ICD	2,913196942	0,014787029
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Epulopiscium.s__2575786	ICD	2,504195664	0,00240333
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Epulopiscium.s__295029	ICD	3,028901583	0,014635653
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Epulopiscium.s__298247	ICD	3,26744118	0,013164598

Table 1 : Characteristics of patients

	IBD flare CDI (n=8)		**IBD flare (n=24)**		**IBD remission (n=24)**		**HS (n=24)**
Age : Year (mean +/- SD)	33.4 +/- 10.5		36.5 +/- 11.6		36.5 +/- 11.6		33.0 +/- 10.2
Male : n (%)	5 (62.6%)		14 (58.3%)		14 (58.3%)		15 (62.5%)
Active smoking : n(%)	2 (25.0%)		9 (37.5%)		9 (37.5%)		1 (4.2%)
CD/UC : n(%)	CD	UC	CD	UC	CD	UC	NA
n (%)	6 (75%)	2 (25%)	18 (75%)	6 (25%)	18 (75%)	6 (25%)	NA
Montreal classification							
A1 / A2 / A3 (n)	6 / 0 / 0	NA	4 / 12 / 2	NA	3 / 15 / 0	NA	NA
L1 / L2 / L3 (n)	2 / 2 / 2	NA	6 / 6 / 6	NA	6 / 6 / 6	NA	NA
B1 / B2 / B3 (n)	4 / 0 / 2	NA	9 / 2 / 7	NA	6 / 2 / 10	NA	NA
E1 / E2 / E3 (n)	NA	0 / 1 / 1	NA	0 / 3 / 3	NA	0 / 3 / 3	NA
Treatment: n(%)							NA
5-ASA	3 (37.5%)		10 (41.7%)		6 (25.9%)		0
Coticosteroids	1 (12.5%)		10 (41.7%)		1 (4.2%)		0
Thiopurine or MTX	1 (12.5%)		10 (41.7%)		10 (41.7%)		0
anti-TNFa	2 (25.0%)		13 (54.2%)		19 (79.2%)		0
Antibiotics	0		0		0		0