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► To cite this version:

Harry Sokol, Sarah Jegou, Claire Mcquitty, Marjolene Straub, Valentin Leducq, et al.. Specificities of the intestinal microbiota in patients with inflammatory bowel disease and *Clostridium difficile* infection. *Gut microbes*, 2017, 9 (1), pp.1-6. 10.1080/19490976.2017.1361092 . hal-01582584

HAL Id: hal-01582584

<https://hal.sorbonne-universite.fr/hal-01582584v1>

Submitted on 6 Sep 2017

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

3
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19

20 Short title: Microbiota in IBD with CDI

21

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

24

25 **Abstract**

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

29 **Methods:** The fecal microbiota of 56 IBD patients, including 8 in flare with concomitant CDI,
30 24 in flare without CDI, and 24 in remission, as well as 24 healthy subjects, was studied using
31 16S sequencing. Analysis was performed using the Qiime pipeline.

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

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

38

39 **Keywords:** IBD, *Clostridium difficile*

40

41 **Introduction**

42 *Clostridium difficile* infection (CDI) is a common complication of inflammatory bowel disease
43 (IBD) and has been associated with a poor IBD outcome¹. CDI typically occurs after a transient
44 microbiota perturbation in the gut. Thus, antibiotic intake is a major risk factor in the general
45 population. In IBD, as the gut microbiota is basally perturbed (i.e., dysbiosis)², CDI can occur
46 even without an antibiotic trigger.

47 Microbiota composition in patients with CDI is imbalanced and characterized by decreased
48 biodiversity^{3,4}, as well as notable increases and decreases in several taxa that are known to be
49 increased and decreased in IBD⁴, respectively. For example, *Bifidobacterium* and members of
50 *Clostridium* clusters IV and XIVa (such as *Faecalibacterium prausnitzii* or *Roseburia intestinalis*)
51 were decreased, while Enterobacteriaceae and *Enterococcus* were increased⁵. However,
52 intestinal microbiota composition in IBD patients with CDI has not been specifically evaluated
53 to date.

54

55 **Results**

56 **Population, microbiota composition and diversity**

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

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

69 As expected, most of the bacteria from both the IBD and HS samples belonged to the phyla
70 Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria (Figure 1D), and the distribution
71 of these phyla in the patients was in agreement with published data⁶. Firmicutes and
72 Bacteroidetes were the most abundant in HS. In IBD patients, particularly those in flare, there
73 was a decrease in Firmicutes (mostly from the Ruminococcaceae family) associated with an
74 increase of Proteobacteria (mostly from the Enterobacteriaceae family ; Figure 1D, Suppl
75 Figure 1C).

76

77 **Differential bacterial composition and altered microbial network in CDI**

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

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

94

95 Discussion

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

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

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

112

113 **Materials and Methods**

114 **Patients and sample collection**

115 All patients were recruited at the Gastroenterology Department of the Saint Antoine Hospital
116 (Paris, France) and provided informed consent. Approval was obtained from the local ethics
117 committee (Comite de Protection des Personnes Ile-de-France IV, Suivitheque study). A
118 diagnosis of IBD was defined by clinical, radiological, endoscopic and histological criteria. A
119 flare was determined by the physician in charge of the patient based on clinical and biological
120 data. None of the study participants reported having taken antibiotics or using colon-cleansing
121 products for at least 2 months prior to enrollment. Patient characteristics are presented in
122 Table 1. Samples from healthy subjects (HS) and from IBD patients without a *Clostridium*
123 *difficile* infection were described previously². Whole stools were collected in sterile boxes and
124 immediately homogenized, and 0.2 g aliquots were frozen at -80°C for further analysis.

125

126 **Fecal DNA extraction**

127 Genomic DNA was extracted from 200 mg of feces as previously described¹. Following
128 microbial lysis with both mechanical and chemical steps, nucleic acids were precipitated in
129 isopropanol for 10 minutes at room temperature, incubated for 15 minutes on ice and
130 centrifuged for 30 minutes at 15,000 *g* and 4°C. Pellets were suspended in 112 µL of phosphate
131 buffer and 12 µL of potassium acetate. After RNase treatment and DNA precipitation, nucleic
132 acids were recovered via centrifugation at 15,000 *g* and 4°C for 30 minutes. The DNA pellet
133 was suspended in 100 µL of TE buffer.

134

135 **16S rRNA gene sequencing**

136 After extraction, total DNA concentration was measured using PicoGreen (Invitrogen), and
137 global 16S gene DNA copy numbers were measured using a qPCR method adapted from
138 Maeda *et al.*¹⁰, enabling inhibition effect estimation and DNA concentration adjustment. The
139 sequence region of the 16S rRNA gene spanning the variable region V3-V5 was amplified using
140 the broad-range forward primer, For16S_519 (CAGCMGCCGCGGTAATAC), and reverse primer,
141 Rev16S_926 (CCGTCAATTCMTTGGAGTTT). The amplification reaction (initial activation step at
142 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
143 incubation step at 68°C for 1 min) was performed in a total volume of 100 µL containing 1X
144 PCR buffer, 2 mM MgSO₄, 1 U of DNA High Fidelity Taq Polymerase (Invitrogen, Carlsbad, CA),
145 625 nM of each barcoded primer (IDT), 250 µM of each dNTP (Invitrogen) and the
146 concentration-adjusted DNA sample. A bidirectional library was prepared using the One
147 Touch2 Template Kit and sequenced on PGM Ion Torrent using the Ion PGM Sequencing 400
148 Kit (Life Technologies, Carlsbad, CA).

149

150 **16S rRNA gene sequence analysis**

151 The sequences were demultiplexed and quality filtered using the Quantitative Insights Into
152 Microbial Ecology (QIIME, version 1.8.0) software package¹¹. The sequences were trimmed for
153 barcodes and PCR primers and were binned for a minimal sequence length of 200 bp. The
154 sequences were then assigned to (OTUs) using the UCLUST algorithm¹² with a 97% threshold
155 pairwise identity and taxonomically classified using the Greengenes reference database¹³.
156 Rarefaction was performed (10,000 sequences per sample) and used to compare OTU
157 abundances across samples. Principal component analyses (PCA) of the Bray Curtis distance,
158 with each sample colored according to disease phenotype, were built and used to assess the
159 variation between experimental groups. The number of observed species, as well as the
160 Shannon, Simpson and Chao1 diversity indexes, were calculated using rarefied data (depth =
161 10,000 sequences/sample) and used to characterize species diversity in a community.

162

163 **Clostridium difficile testing**

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

168

169 **Statistical analysis**

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

174 the beta diversity analysis was performed using the Permanova method (9999 permutations).
175 Differences with a P value less than 0.05 were considered to be significant.
176 Comparisons between IBD patients with and without CDI was performed using the linear
177 discriminant analysis (LDA) effect size (LEfSe) pipeline ⁷.
178 A correlation network was built using an OTU table and the Spearman correlation. Only OTUs
179 present in >50% of the samples were used. The p values were corrected using the Benjamini
180 and Hochberg procedure to control for the false discovery rate (FDR). Correlation network
181 figures were built using Cytoscape 3.1.1. The relative connectedness of the networks was
182 calculated by dividing the number of significant interactions (edges) by the number of taxa
183 (nodes) in the network. Statistical significance was assessed by a chi-square test.

184

185 **Authors' Contributions**

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

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

191

192 **Funding**

193 This study was supported by an unrestricted Biocodex grant.

194

195

196 **Conflicts of interest**

197 The authors disclose no conflicts of interest.

198

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246

247

248 **Figure Legends**

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

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

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Supplementary Materials and Methods

271

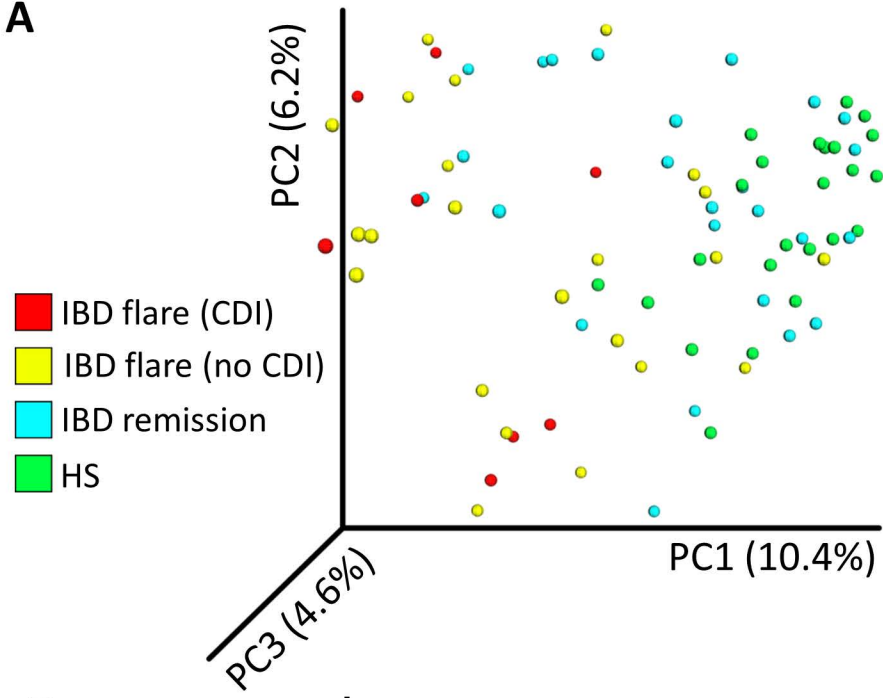
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274 **Supplementary Figure Legend**

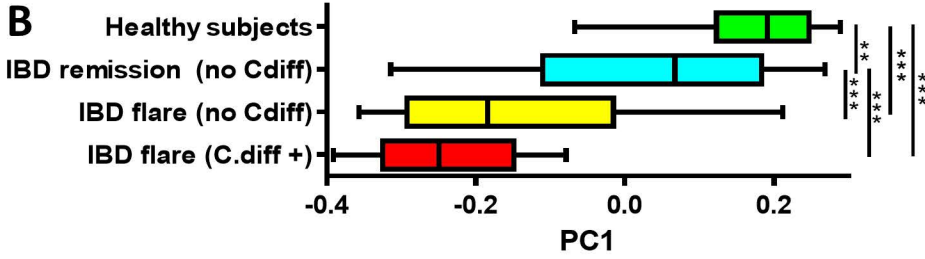
275 **Supplementary Figure 1: Bacterial microbiota biodiversity and composition.** (A) Chao1
276 diversity index of the bacterial microbiota in the different groups studied. (B) Shannon
277 diversity index of the bacterial microbiota in the different groups studied (t test, * = $p < 0.05$;
278 ** = $p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

281

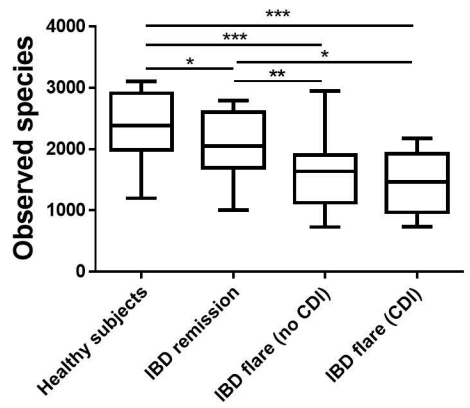
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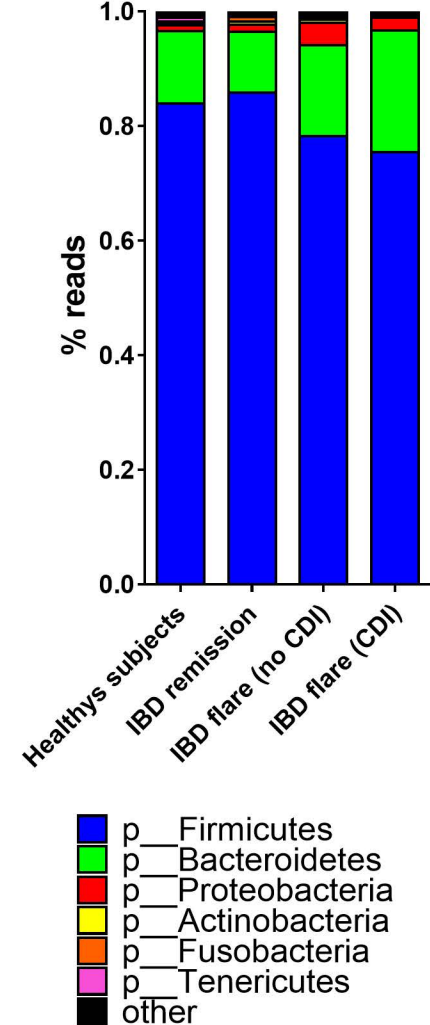
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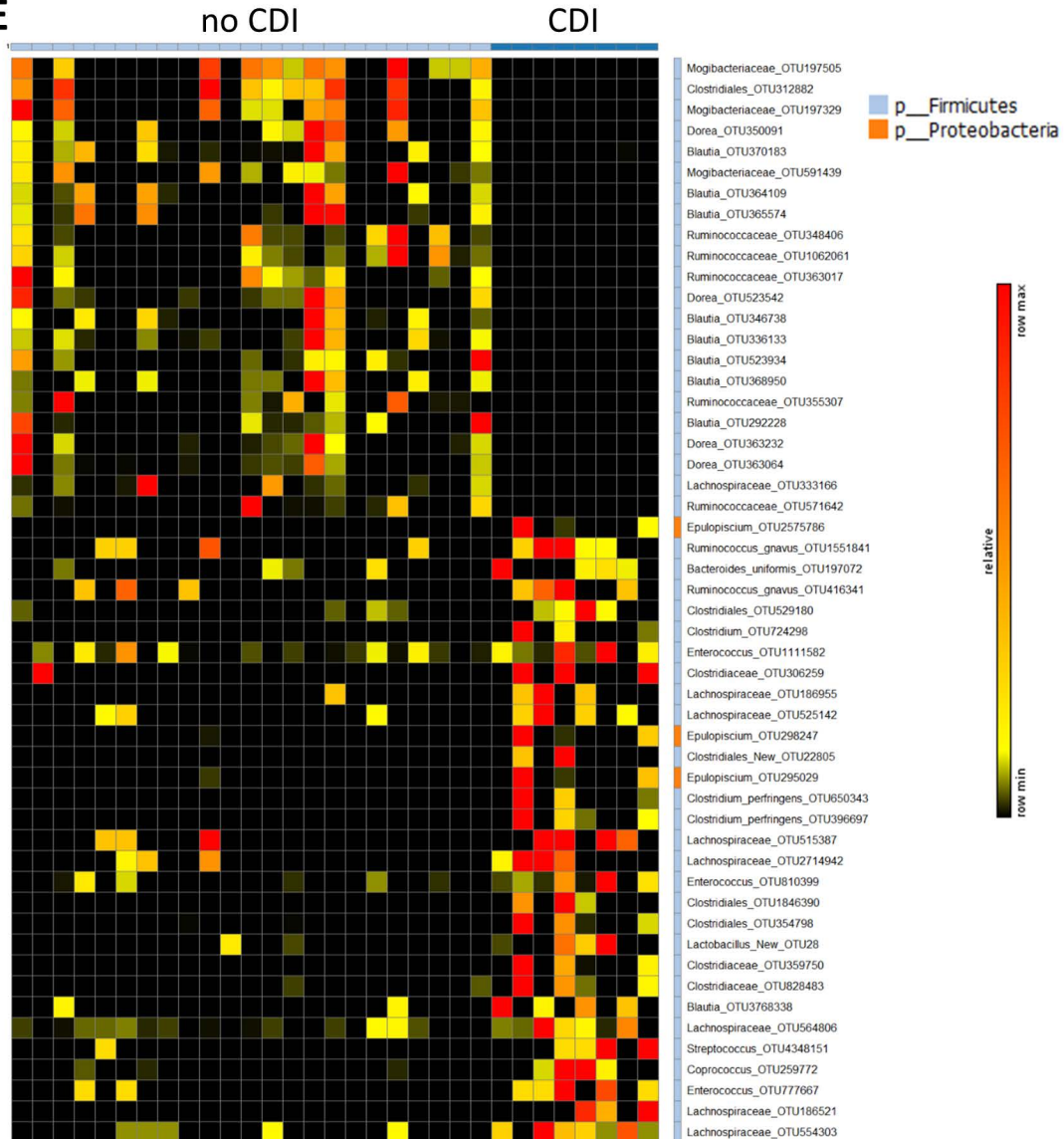
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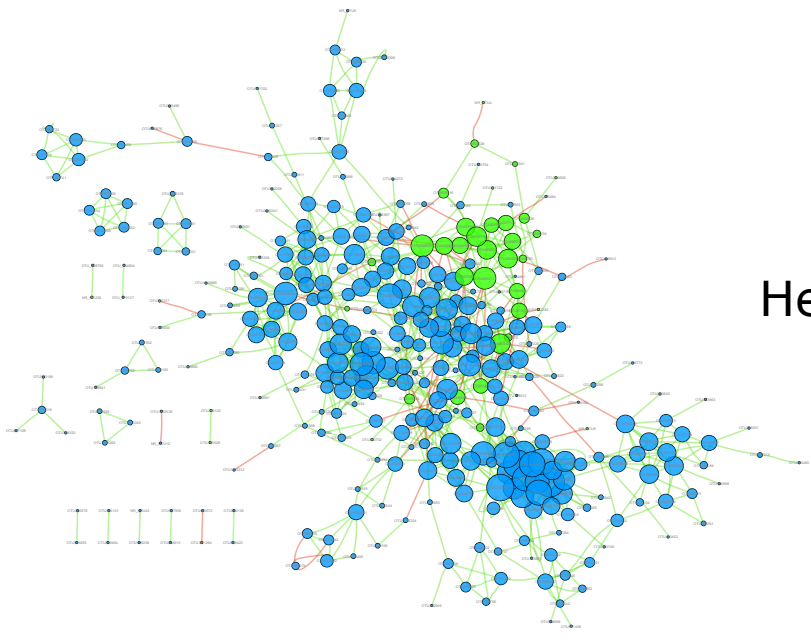
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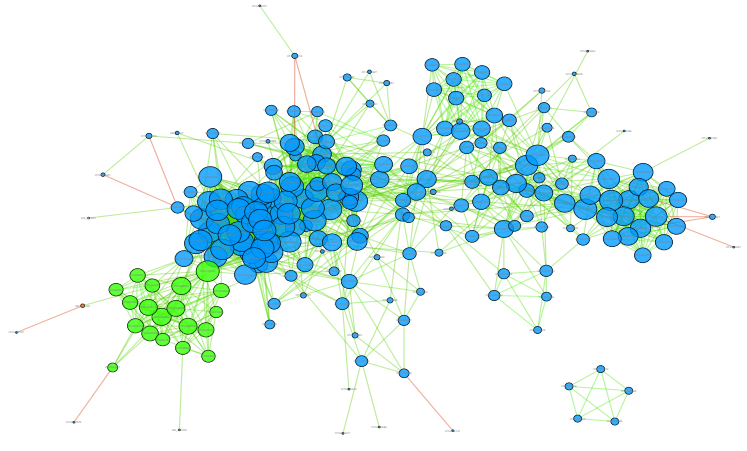
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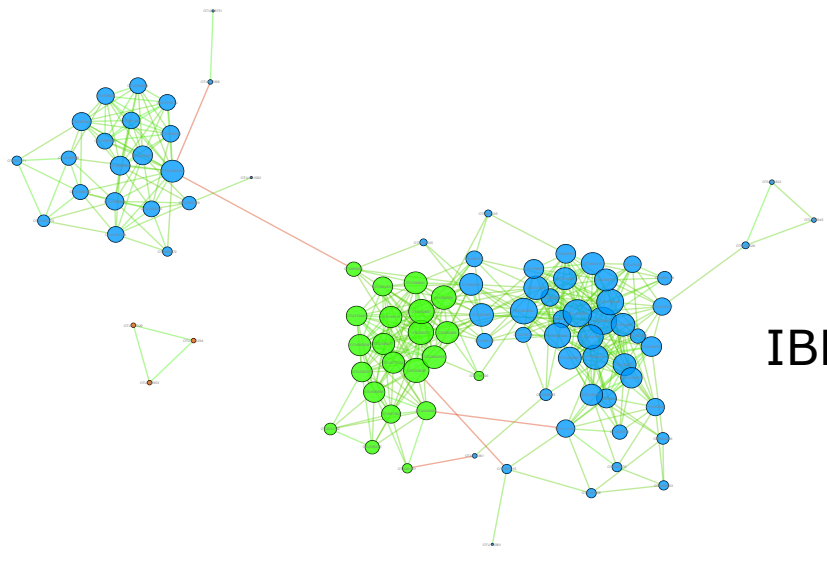
Healthy subjects



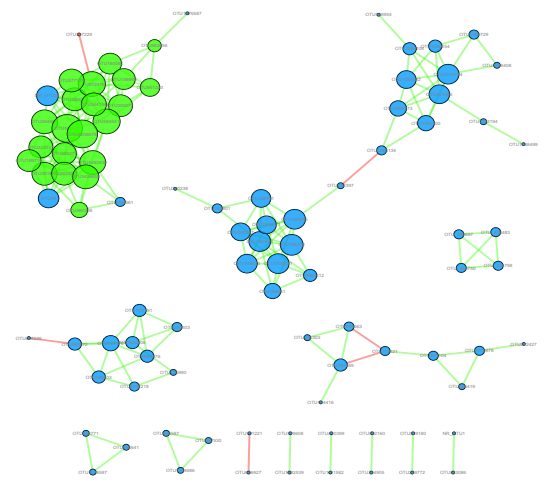
IBD remission

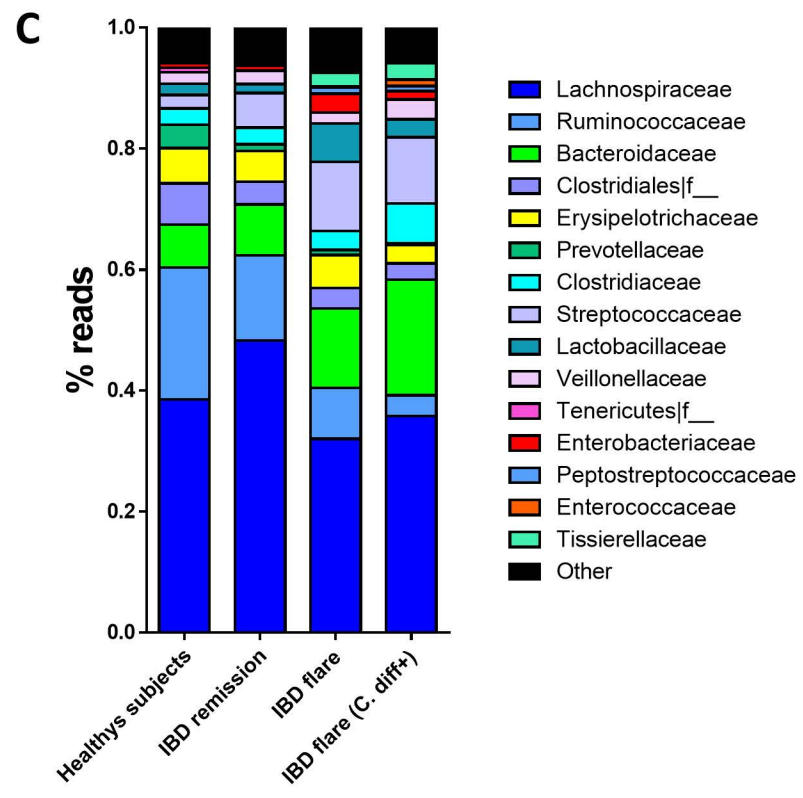
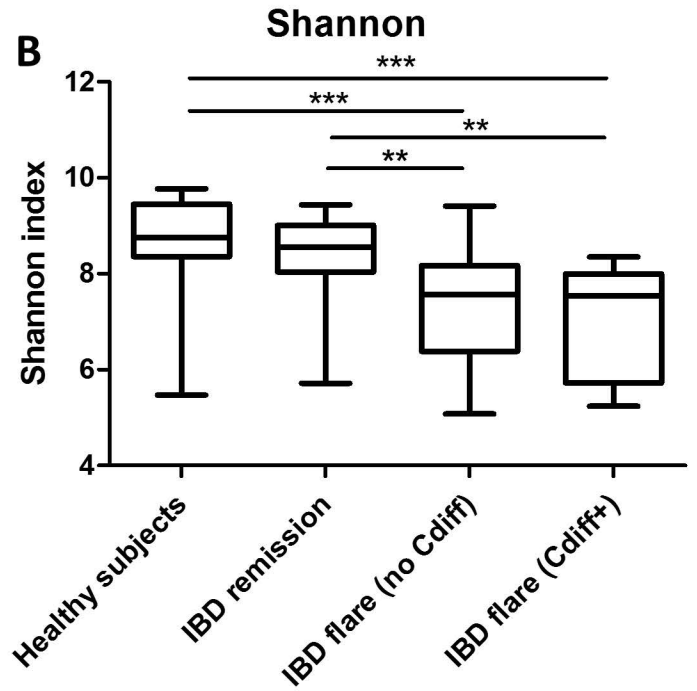
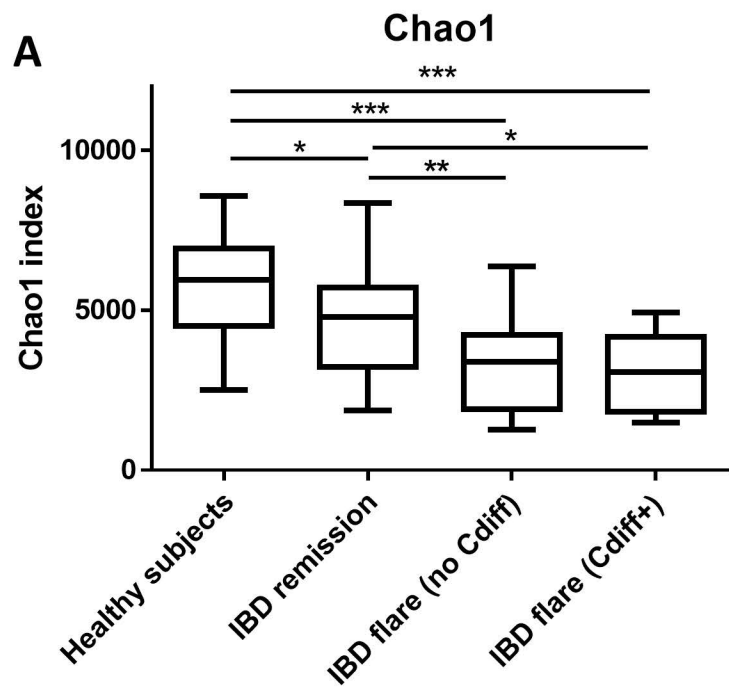


IBD flare (no CDI)



IBD flare (CDI)





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
Corticosteroids	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