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**Gut microbiota differs between children with inflammatory bowel  
disease and healthy siblings in taxonomic and functional composition:  
a metagenomic analysis**

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This is a copy of the accepted manuscript, as originally published online ahead of print by the American Physiological Society. The original article has been published in final edited form in:

American Journal of Physiology Gastrointestinal and Liver Physiology  
2017 APR 01 ; 312(4): G327-G339  
2017 APR 01 (first published online)  
doi: [10.1152/ajpgi.00293.2016](https://doi.org/10.1152/ajpgi.00293.2016)

Publisher: [American Physiological Society](http://www.americanphysiological.org)

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1 **Gut microbiota differs between children with Inflammatory Bowel**  
2 **Disease and healthy siblings in taxonomic and functional composition**  
3 **- a metagenomic analysis**

4 **Running Title: Metagenomic analysis of the pediatric IBD microbiome**

5

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28

29 **Abstract**

30 Current treatment for pediatric IBD patients is often ineffective, with serious side effects.  
31 Manipulating the gut microbiota via fecal microbiota transplantation (FMT) is an emerging  
32 treatment approach but remains controversial. We aimed to assess the composition of the fecal  
33 microbiome through a comparison of pediatric IBD patients to their healthy siblings, evaluating  
34 risks and prospects for FMT in this setting. A Case–Control (Sibling) Study was conducted  
35 analyzing fecal samples of six children with Crohn’s Disease (CD), six children with Ulcerative  
36 Colitis (UC) and 12 healthy siblings by metagenomic sequencing. In addition, lifetime antibiotic  
37 intake was retrospectively determined. Species richness and diversity were significantly reduced in  
38 UC patients compared to control (MWU FDR = 0.011). In UC, bacteria positively influencing gut  
39 homeostasis e.g. *Eubacterium rectale* and *Faecalibacterium prausnitzii* were significantly reduced  
40 in abundance (MWU FDR = 0.05). Known pathobionts like *Escherichia coli* were enriched in UC  
41 patients (MWU FDR = 0.084). Moreover, *E. coli* abundance correlated positively with that of  
42 several virulence genes (SCC > 0.65, FDR < 0.1). A shift towards antibiotic resistant taxa in both  
43 IBD groups distinguished them from controls (MWU BY FDR = 0.062 in UC, MWU BY FDR =  
44 0.019 in CD). The collected results confirm a microbial dysbiosis in pediatric UC, and to a lesser  
45 extent in CD patients, replicating associations found previously using different methods. Taken  
46 together, these observations suggest microbial remodeling therapy from family donors, at least for  
47 children with UC, as a viable option.

48 **New and Noteworthy**

49 In this sibling study, prior reports of microbial dysbiosis in IBD patients from 16S rRNA  
50 sequencing was verified using deep shotgun sequencing and augmented with insights into the  
51 abundance of bacterial virulence genes and bacterial antibiotic resistance determinants, seen against  
52 the background of data on the specific antibiotic intake of each of the study participants. The  
53 observed dysbiosis, which distinguishes patients from siblings, highlights such siblings as potential  
54 donors for microbial remodeling therapy in IBD.

55

56 **Keywords:** Metagenomics, Microbiome, pediatric gastroenterology, Inflammatory Bowel Diseases, Fecal

57 Microbiota Transplantation (FMT)

58

59 **Abbreviations:**

BY FDR	Benjamini-Hochberg-Yekutieli procedure False Discovery rate (adjustment)
CD	Crohn' s Disease
CTRL	Control
(BH) FDR	Benjamini-Hochberg procedure False Discovery Rate (adjustment)
FMT	Fecal Microbiota Transplantation
GABA	gamma-Aminobutyric acid
IBD	Inflammatory Bowel Disease
KW	Kruskal-Wallis test
LRT	likelihood ratio test
mOTUs	metagenomic Operational Taxonomic Units
MWU	Mann-Whitney-U test
PCDAI	Pediatric Crohn's Disease Activity Index
PUCAI	Pediatric Ulcerative Colitis Activity Index
rRNA	ribosomal RNA
SCC	Spearman rank Correlation Coefficient
UC	Ulcerative Colitis

60

61 **Introduction**

62 The gut microbiota plays a crucial role in human physiology and host development (41), and  
63 maintaining equilibrium between the commensal microbiota and the host immune system is  
64 required for a healthy gut homeostasis.

65 There is increasing evidence for a contribution of the gut microbiome to the etiology of IBD (18). In  
66 the past, genome-wide association studies have revealed multiple host gene loci in both UC and  
67 CD, with alleles associated with functional aberrations of the intestinal immune system (18). Recent  
68 studies based on novel DNA sequencing methods have revealed major differences in bacterial  
69 taxonomic composition between IBD patients and healthy individuals (see Appendix Table 1). Still,  
70 it remains unclear whether these observed alterations are the cause or result of inflammation.

71 Since most studies on the IBD microbiome to date have relied on 16S rRNA sequencing, the  
72 functional impact of the reported dysbiosis is not well understood (23), and reported associations  
73 are largely limited to identifying more general taxa (ranging from bacterial phyla to genera) as  
74 being associated with disease, given the limitations of 16S for reliable species identification. Whole  
75 genome sequencing allows higher resolution and sensitivity than the more common and less  
76 expensive 16S rRNA sequencing and offers new insights into the functional context of the IBD  
77 microbiome (e.g. abundance of metabolic pathways and the distribution of genes determining  
78 virulence or resistance to antibiotics).

79 Current treatment strategies for pediatric IBD patients often come with serious side effects or  
80 provide insufficient treatment responses. Therefore, there is a need for novel treatment approaches.

81 Manipulating the gut microbiota via fecal microbiota transplantation (FMT) appears as an  
82 intriguingly facile and harmless therapy option for children with IBD. Efficacy in the treatment of  
83 pediatric UC via fecal enemas has been suggested (22), and the administration of FMT via  
84 nasogastric tube has led to improved well-being in pediatric CD patients (44). Still, its outcome  
85 remains controversial, since results from the first two randomized placebo-controlled trials in adults

86 are in contradiction (29, 36). While no serious adverse events for FMT in children have been  
87 reported, data on feasibility and safety in a long-term perspective (e.g. the risk of transferring a  
88 pathogenic disease state via FMT) are missing (12). Microbiome-based validation of suitable donors  
89 might help better predict treatment outcome (11). The present pilot study therefore aims to elucidate  
90 microbiome correlates of juvenile IBD so as to help in designing criteria for when FMT might be  
91 employed, using a pediatric cohort of CD and IBD patients as well as their healthy siblings.

## 92 **Methods**

### 93 *Cohort recruitment*

94 Patients were selected from the IBD patient collective at the Zentrum für Kinder- und  
95 Jugendmedizin (University Children's Hospital) Mainz in Mainz, Germany. In all patients IBD  
96 diagnosis was histologically confirmed by prior endoscopy. Disease activity was assessed by the  
97 Pediatric Ulcerative Colitis Activity Index (PUCAI) (45) and the Pediatric Crohn's Disease Activity  
98 Index (PCDAI) (14). In total, fecal samples from six Ulcerative Colitis (UC) patients and six  
99 Crohn's Disease (CD) patients and from 12 controls were collected from August 2013 to July 2014.  
100 Controls were healthy siblings of the IBD patients, sharing the same parents and living in the same  
101 households, thereby sharing genetic background, environment and diet. In addition, study  
102 participants completed a questionnaire about health/disease status, living conditions, and alimentary  
103 habits. Children who had taken antibiotics during the last 2 months prior to sampling were excluded  
104 from the study. For additional cohort characteristics see Table 1. Since all study participants are  
105 older than 8 years, and thus should have relatively stable microbiomes, we do not anticipate age  
106 differences between patients and healthy siblings to have a strong impact on results.

107 The study design was approved by the ethics committee of the State Chamber of Physicians of  
108 Rhineland-Palatinate (Ethikkommission der Landesärztekammer Rheinland-Pfalz, reference number  
109 837.292.13 (8977-F)).

110 Written informed consent was given by both parents and the study participants prior to sample  
111 collection and medical assessment.

#### 112 *Assessment of lifetime antibiotic intake*

113 Antibiotic intake of the study population was retrospectively recorded, starting from birth.  
114 Therefore, all physicians/hospitals who have treated the study participants were contacted and asked  
115 to transfer their antibiotic prescription data for each child. Out of 306 person years of total  
116 participant lifetime to date, only 16 (5 %) could not be covered by this assessment.

#### 117 *Sample collection*

118 Fecal samples were collected at home with help of a *stool sampling kit*. The *stool sampling kit*  
119 consisted of a plastic lining to cover the toilet, two stool sample tubes with spoons, two plastic bags  
120 and a clipping system for safe closure of the outer bag. Collected samples were stored at home  
121 between 4°C and 8°C and transferred to the laboratory within 24h. In the laboratory of the Zentrum  
122 für Kinder- und Jugendmedizin Mainz, Germany, the samples were frozen at – 80°C. All samples  
123 were gathered there and then shipped on dry ice (- 78.5°C) to the European Molecular Biology  
124 Laboratory (EMBL) in Heidelberg, Germany.

#### 125 *Probe processing*

126 All probe processing was conducted by the Genomics Core Facility, EMBL, Heidelberg. DNA-  
127 Extraction and Library preparation were performed according to the protocol from Zeller et al. (49).  
128 Whole genome shotgun sequencing was executed on the Illumina HiSeq 2000/2500 (Illumina, San  
129 Diego, USA) platform. All samples were paired-end sequenced with a read length of 100 bp and a  
130 targeted sequencing depth of 5 Gbp (49).

#### 131 *Data analysis*

132 Data analysis was performed at the Structural and Computational Biology Unit, EMBL, Heidelberg.

#### 133 *Taxonomic profiling*

134 Using the MOCAT pipeline (21), gene sequences were annotated to their bacterial taxonomy. Since  
135 many gut bacteria are known to belong to species for which no genome yet exists in public  
136 databases, two different alignment procedures were used. The first procedure was based on  
137 metagenomic operational taxonomic units (mOTUs) (43) which also encompass uncharacterized  
138 bacteria identified in metagenomic dataset. The second procedure used species clusters defined  
139 from bacterial genomes for which publicly deposited genomes do exist (27). In this manner as  
140 complete coverage of the taxonomic composition of the samples as possible was achieved.

#### 141 *Analysis of bacterial diversity, species richness and evenness*

142 Based on the mOTU taxonomic composition, Shannon diversity index, species richness and  
143 evenness were calculated using the vegan R package ([http://cran.r-](http://cran.r-project.org/web/packages/vegan/index.html)  
144 [project.org/web/packages/vegan/index.html](http://cran.r-project.org/web/packages/vegan/index.html)), for details see Zeller et al. 2014 (49).

#### 145 *Functional profiling*

146 To gain insight into metabolic functions of the microbiome, the metagenomic catalogue was aligned  
147 to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Database (16). For further details see  
148 Zeller et al., 2014 (49).

#### 149 *Identifying virulence factors*

150 For virulence factor profiling of the metagenome samples, reads were mapped to virulence factor  
151 gene families via an annotated gene catalog as described in Kultima et al., 2016 (20). For each gene  
152 catalog entry annotated to a virulence factor gene family, we traced which sequenced taxa have  
153 genes similar enough to the reference that reads originating from them could map to it. For the  
154 virulence genes found significantly different under IBD in this study, from sequence alone their  
155 specific origin cannot be determined; on average each virulence gene family is found in 87 taxa  
156 (See Supplementary Table 2).

#### 157 *Identifying antibiotic resistance genes*



158 To estimate the abundance and prevalence antibiotic resistance genes in the studied microbiomes,  
159 the reference gene catalogue was aligned to the Antibiotic Resistance Genes Database (ARDB)(25),  
160 as described in Forslund et al. (9). Metronidazole resistance genes (nim, nimA, nimB, nimC, nimD,  
161 nimF) were further annotated through bidirectional best hits between the reference gene catalog and  
162 all annotated such sequences from UniProt, otherwise as previously described. Only antibiotic  
163 classes which had been taken by at least one individual in the study cohort were considered.

164 For each sample, three measures of antibiotic resistance gene carriage were determined: the raw  
165 relative abundance of antibiotic resistance genes, the relative abundance of potentially resistance  
166 gene-carrying bacterial species, and the antibiotic resistance potential (abundance of antibiotic  
167 resistance genes relative to the abundance of potentially resistance gene-carrying species). The  
168 abundance of potentially resistant species was calculated as the fraction of genetic material from  
169 each sample that map to species belonging to genera with known examples of species carrying  
170 resistance genes of the appropriate type, in the reference genome database used (see Forslund et al.  
171 (9)).

#### 172 *Statistical analysis*

173 In subsequent analyses, all read/base counts were transformed into relative abundances (by division  
174 by the total number of reads/bases sequenced per sample) as described previously by Zeller et al.,  
175 2014 (49).

176 In order to identify significant differences between sample categories for each metagenomic feature,  
177 a nonparametric Kruskal-Wallis-test (KW) was performed whenever the UC-, CD- and control-  
178 group were compared. This was followed by pairwise Mann-Whitney-U-tests (MWU) as a post-hoc  
179 procedure.

180 For data sets not normally distributed, the Wilcoxon signed-rank test was employed to compare sib  
181 pairs directly. Correlation analyses were performed using Spearman rank's correlation coefficient  
182 (SCC), in order to fit potentially non-normal data.

183 In cases of multiple testing p-value correction via the Benjamini-Hochberg false discovery rate  
184 (FDR)(2) or the Benjamini-Hochberg-Yekutieli procedure (BY) (3) was performed. For corrected p-  
185 values significance threshold was set at  $< 0.5$ ; corrected p-values ranging  $0.05 < \text{FDR} < 0.1$  we refer  
186 to as approaching significance.

187 To reduce the number of tests for taxonomic abundances and thereby increase statistical power, a  
188 pre-selection of hypotheses was applied by collecting microbiome associations described in the  
189 literature, including taxa directly associated with IBD or associations projected from such reports by  
190 phylogenetic relationships. This collection consisted of all directly reported IBD-associated taxa in  
191 the referenced works and their containing taxonomic superclades (see Appendix Table 1). In  
192 addition, thus far uncharacterized taxa sorting immediately under each clade previously reported  
193 associated with IBD were added, to account for incompleteness of sequenced genomes (e.g.  
194 unknown Firmicutes for class analysis, as Firmicutes were reported to be different on the Phylum  
195 level, or unknown Clostridia for analysis on the order level, as Clostridia were previously reported  
196 to be associated with IBD on the class level); the mOTU technology used here was explicitly  
197 designed to allow detection of unculturable human gut taxa.

198 A likelihood ratio test (LRT) was conducted to test whether the abundance of potentially resistant  
199 species can be explained by antibiotic selection pressure (as represented through lifetime antibiotic  
200 intake) or not. To calculate these likelihood ratios, mixed-effect linear models taking into account 1)  
201 overall lifetime antibiotic intake and disease status, 2) lifetime antibiotic intake alone, 3) disease  
202 status alone and 4) solely a background constant model were compared to each other. Prior to this  
203 analysis in each case the general assumptions for linear models were tested (*independence, absence*  
204 *of co-linearity, homoscedasticity, linearity and normality of the residuals and absence of influential*  
205 *data points*) using graphpad (<http://graphpad.com/quickcalcs/PValue1.cfm>) with p-values calculated  
206 for a  $\chi^2$ -distribution with one degree of freedom.

## 207 **Results**

208 *Pediatric UC patients have reduced microbial biodiversity compared to their healthy siblings*

209 Tests were conducted on the Shannon diversity index to characterize overall gut biodiversity. Only  
210 in UC patients reduction of biodiversity reached significance compared to controls (MWU,  $n_{UC} =$   
211  $6/n_{Control} = 12$ , FDR = .011) (Figure 1). Analyzing the data on aggregate, children with UC and CD  
212 both displayed significantly reduced species richness (MWU,  $n_{UC} = 6/ n_{CD} = 6/ n_{Control} = 12$ , FDR =  
213 .011, FDR= .045 respectively) (Figure 1) compared to the set of control children. This reduction  
214 was significant also when matching each IBD patient to their control sibling in a pairwise test (WT,  
215  $n_{UC} = 6/ n_{CD} = 5/ n_{Control} = 11$ , FDR= .048). Similar observations could be made for microbial  
216 community evenness (see Figure 1).

217 *Differences in taxonomic abundances from former 16S rRNA analyses are validated by WGS*

218 Considering those bacterial taxa previously noted as being altered in prevalence or abundance in  
219 IBD (cf Appendix Table 1), many of these findings could be recaptured as significant in the present  
220 cohort even with its limited size, comparing the set of all controls to the set of each subset of IBD  
221 subjects (Figure 2). Pairwise testing of siblings (Wilcoxon rank test) showed the same trends as the  
222 aggregated case-control comparison, though due to limited sample numbers, approached  
223 significance only for associations of *Clostridium* (Clostridiaceae) and *Eubacterium rectale* to IBD  
224 (Supplementary Table 1).

225 If we do not restrict analysis to validation of 16S results only, power is reduced due to the increased  
226 number of tested hypotheses. However, 55% (17 out of 31) of the findings described above also  
227 show associations with IBD approaching significance without such preselection and with  
228 subsequently more stringent FDR correction (Supplementary Table 3). The previously described  
229 associations constitute the majority of significant associations found in the unrestricted search,  
230 suggesting most obvious associations already were reported at least once from 16S data. However,  
231 another four taxa approached significance in the unrestricted test, which had not previously been  
232 linked to IBD. On the taxonomic order level, a group of uncharacterized Firmicutes were

233 diminished in the UC population compared to the healthy cohort. At the level of bacterial genera,  
234 *Anaerococcus* (MR<sub>(Control)</sub> 10.83, MR<sub>(UC)</sub> 18.33, MR<sub>(CD)</sub> 10.00, KW FDR = .093) and a thus far  
235 uncharacterized genus belonging to Clostridiales (MR<sub>(Control)</sub> 17.08, MR<sub>(UC)</sub> 5.83, MR<sub>(CD)</sub> 10.00,  
236 KW FDR = .093), differed in abundance between IBD subjects and controls. Most importantly,  
237 *Clostridium ramosum* was significantly enriched in UC patients compared to the set of controls  
238 (FDR <.001).

### 239 *Enrichment of pathobiont species and depletion of commensals*

240 Reduction of gut homeostasis positively influencing *E. rectale* and *Faecalibacterium prausnitzii*  
241 were observed in UC and CD samples compared to the set of controls samples, but only the  
242 comparison between control and UC samples approached significance (Figure 2). Similarly, the  
243 pathobiont *Escherichia coli* was increased in abundance in both IBD conditions, but approached  
244 significance only for UC samples (Figure 2). An increase in *Ruminococcus gnavus* approached  
245 significance (at the species cluster level, not significant at mOTU level). *Bilophila wadsworthia* was  
246 decreased in UC, also approaching significance. The pathogen *Fusobacterium nucleatum* (resolved  
247 at the species cluster level, not significant at mOTU level) was found in IBD patients but not in the  
248 healthy siblings, with this difference approaching significance for UC patients (Figure 2).

### 249 *The abundance of specific virulence factors correlates with the abundance of certain bacterial* 250 *species*

251 Figure 3 gives an overview of the specific virulence genes, which were significantly increased in  
252 the UC population (CD and UC samples compared to the full set of control samples) of the present  
253 study. To identify correlations with species present in the study cohort, a Spearman correlation  
254 analysis was conducted. Most virulence factors were correlated (Spearman test FDR < 0.1) with  
255 abundance of *E. coli*. All of these genes, except hslT, were previously described and identified as  
256 occurring in strains of *E. coli* (see Supplementary Table 2).

257 Abundance of certain families of genes facilitating the survival of bacteria within macrophages and  
258 the evasion of immune response correlated with abundance of particular gut microbial species  
259 (*Bacteroides vulgatus/Gemella morbillorum*). Virulence genes adhD, aslA, sitA, Ndk, fur, gcvT and  
260 fepA had no significant correlation with any species found significantly different in abundance  
261 between controls and IBD patients. These observations suggest a broader involvement of those  
262 genes in UC pathogenesis, such that they may play a role which is not reducible to a simple  
263 taxonomic difference.

#### 264 *Abundance of GABA shunt genes is elevated in the IBD microbiome*

265 A Kruskal-Wallis test was performed to analyze KEGG pathways and KEGG modules for  
266 significant differences in gene abundance comparing all IBD cases to all controls. Only the KEGG  
267 module representing the GABA (gamma-Aminobutyric acid) shunt, considered a potential virulence  
268 factor by some investigators (8), showed such a significant difference with a mean rank of 6.67 for  
269 the Control group, 18.67 for UC and 18.00 for CD; BY <.000.

#### 270 *Little difference in antibiotic intake*

271 Neither the cumulative lifetime antibiotic intake per person nor the mean antibiotic intake per life  
272 year was significantly different comparing the full groups of control, UC and CD.

273 In all three groups cephalosporin was the most commonly prescribed antibiotic, followed by  
274 penicillin, with highest intake for both in the CD group (Figure 4). Metronidazole was only taken  
275 by IBD patients, and a tetracycline antibiotic was only taken once by one subject in the control  
276 group.

#### 277 *The resistome of IBD patients and their healthy siblings*

278 In accordance with previous studies (9), tetracycline was the antibiotic for which the most  
279 resistance capacity was found in the microbiomes of the study cohort (Figure 5), followed by  
280 cephalosporin. No significant difference in the abundance of resistance genes against any antibiotic

281 class was observed comparing the full groups of control, UC and CD samples on aggregate (See  
282 Appendix Figure 1). While we augment the ARDB antibiotic resistance gene database with known  
283 resistance genes for metronidazole, the antibiotic most commonly prescribed to IBD patients, such  
284 genes are still largely uncharacterized. As a result, it is possible we fail to observe an association  
285 between such resistance and metronidazole exposure history.

286 The high relative fraction of potentially resistant bacterial species does distinguish the gut  
287 microbiome of IBD patients from the microbiomes of controls. Higher abundances of bacterial  
288 species where strains with resistance genes have been sequenced were observed for all antibiotic  
289 classes (BY FDR = 0.062 for UC; BY FDR = 0.019 for CD) (Fig. 5, see also Appendix Figure 1).

290 To evaluate whether this high abundance of potentially resistant species was due to antibiotic  
291 selection pressure, a likelihood ratio test was performed testing fits of the data to nested mixed-  
292 effects linear models. The total abundance of potentially resistant species was modeled from overall  
293 lifetime antibiotic intake of each individual together with disease status (Control, UC, CD). A model  
294 including both antibiotic intake and disease status did not fit significantly better than a model  
295 including group affiliation alone (LRT test,  $P > 0.4$ ). We therefore at this point cannot refer the  
296 greater relative prevalence of potentially resistant microbial taxa in the IBD patients than in their  
297 healthy siblings' microbiomes to any higher lifetime antibiotic consumption in these children.

## 298 **Discussion**

299 Our results from comparatively analyzing the microbiome of pediatric IBD patients constitute a  
300 deep sequencing-based consolidated validation of findings from previous research which relied  
301 mostly on 16S rRNA sequencing (see Appendix Table 1 and Supplementary Table 1). To our  
302 knowledge, this is the first study which contrasts metagenomic analysis of antibiotic resistance  
303 genes in relation to lifetime antibiotic intake of study participants in an IBD cohort. We also provide  
304 the first direct metagenomic functional profiling of pediatric IBD samples, including analysis of  
305 virulence factor differential abundance in cases versus controls. By a study design using as controls

306 healthy siblings of IBD participants sharing similar diet and living circumstances, we are able to  
307 minimize potential confounding factors. In addition, although we expected higher antibiotic  
308 consumption within the patient population, which could have provided an alternate interpretation of  
309 the observed dysbiosis, antibiotic intake was not significantly higher among pediatric IBD patients  
310 than other children.

311 Dysbiosis of the intestinal microbiome may involve reduced microbial diversity, enrichment of  
312 potentially pathogenic taxa and/or depletion of beneficial microbiota (31). Microbial dysbiosis has  
313 been observed in numerous disease conditions, e.g. colorectal carcinoma (40), hypertension (48),  
314 psoriatic arthritis (37), diabetes mellitus type 1 (42) and type 2 (17) and especially in pediatric IBD  
315 (see Appendix Table 1).

316 Several research groups observed how microbial diversity and the amount of beneficial commensals  
317 is reduced in the microbiome of both pediatric IBD conditions (10, 28, 39). At the same time,  
318 potential pathobionts like *E. coli* are more prevalent in the IBD microbiome. These findings are  
319 quantifiable using the present setup and we were able to validate them. While in the present study,  
320 we find more microbiome associations of UC than of CD, previous studies found stronger such  
321 associations to CD (see Appendix Table 1 and Supplementary Table 1). As we observe similar  
322 trends in both IBD subtypes for most comparison, we interpret this as that our small sample size  
323 may make us underpowered to detect many of the associations to CD, which larger studies using  
324 similar methodology may be able to recover.

#### 325 *Decrease of gut homeostasis-promoting species in the IBD microbiome*

326 The gut homeostasis-promoting bacterial species *F. prausnitzii* and *E. rectale* were depleted in both  
327 IBD conditions in the present cohort. This observation is in consensus with former microbiome  
328 analysis of UC and CD patients relying on 16S rRNA sequencing (6, 10, 26). Both species are  
329 important producers of short-chain fatty acids (SCFA), including propionate and butyrate (34).

330 SCFAs are among the most central metabolites produced by microbes, influencing both colon  
331 physiology and the intestinal immune system (30).

### 332 *Increase of E. coli and F. nucleatum in pediatric IBD*

333 In contrast, pathobionts such as *E. coli* and *F. nucleatum* were enriched in the IBD microbiome.

334 This is in agreement with former 16S rRNA analyses, where *E. coli* was increased in the IBD

335 microbiome (6, 28, 39). *E. coli*, especially its subspecies *adherent-invasive E. coli (AIEC)*, has been

336 directly implicated in IBD development (23). A strong correlation was observed between *E. coli*

337 abundance and that of genes implicated in the expression of fimbriae or leading to the adhesion of

338 bacteria to the intestinal mucosa. Those genes are characteristic of pathogenic *E. coli* subspecies

339 (e.g. *enterohaemorrhagic, uropathogenic E. coli*) (1, 5). However, most of these genes are found

340 also in many other bacterial taxa, and as such it cannot be ruled out that other bacterial species also

341 may contribute to the observed increase of virulence capacity in IBD patients (see Supplementary

342 Table 2).

343 The strong correlation of the abundance of virulence genes enriched in the UC microbiome with *E.*

344 *coli* abundance suggests that this species is a central driver of a functional shift towards virulence in

345 UC patients of the present cohort, underscoring its potential role as an IBD partial cause.

### 346 *Survival strategies of bacteria – relating virulence capacity to pathology*

347 The virulence genes enriched in the UC microbiome included TonB, sitA, IroN, fepA and Fur. They

348 are all genes implicated in metal acquisition of Enterobacteria, where the last is the key regulator of

349 iron transport systems (33). The enriched iron uptake/transport systems contribute to the virulence

350 of their host bacteria by counteracting the so called nutritional immunity which deprives bacteria of

351 iron. The homeostasis of intracellular iron concentrations is maintained by Fur (the ferric uptake

352 regulator), since high intracellular accumulation also is toxic for the bacteria (32). In its role as a

353 sensor for iron availability, Fur is also able to regulate directly or indirectly (through iron

354 concentration) the expression of several other virulence factors in pathogenic bacteria.



355 Adaptation of microbes in IBD to a lower environmental pH, as a feature of inflammation, is  
356 reflected by higher abundance of the genes encoding the GABA shunt module in the IBD  
357 microbiomes studied here (Figure 4B). The GABA shunt supplies bacteria with nitrogen and  
358 promotes their survival in acidic conditions and other environmental stresses (8). Feehily and  
359 Karatzas therefore suggested that this module could facilitate microbial pathogenicity.

#### 360 *Exploration of the IBD resistome in correlation with antibiotic consumption*

361 Several studies have displayed a significant relation of antibiotic intake in childhood with the later  
362 development of IBD, particularly Crohn's Disease (13, 19, 47). Analogously, antibiotic  
363 consumption often exacerbates dysbiosis, seen as a reduced species richness and the bloom of  
364 pathogens (e.g. *Clostridium difficile*) in the gut microbiome (46). In addition, administration of  
365 antibiotics not only leads to shifts in taxonomic composition, but also induces the lateral transfer  
366 and spread of antibiotic resistance genes in a microbial ecosystem (38).

367 Notably, the quantification of antibiotic resistance genes in the microbiomes of the present cohort  
368 revealed no significant difference in the relative abundance of these genes between IBD patients  
369 and their healthy siblings, and similar retrospective assessments of antibiotic intake in both groups.  
370 In other words, while it seems unlikely the IBD discordance within the sib pairs can be explained by  
371 differences between siblings in antibiotic exposure, the possibility remains that the families  
372 themselves differ from other families in both antibiotic exposure and IBD prevalence, highlighting  
373 the inter-sibling differences in microbiome composition as rather reflecting IBD risk keeping  
374 antibiotic exposure constant. It is further conceivable that resistant bacteria acquired from intra-host  
375 adaptation to antibiotic treatment or from healthcare setting exposure may propagate between  
376 family members living in close contact. In contrast, a significant shift in both IBD conditions  
377 towards a higher abundance of species potentially carrying antibiotic resistance genes was revealed.  
378 This measure could not be reliably predicted from individual antibiotics use history within the  
379 present dataset, however.

380 Besides antibiotic treatment, intake of IBD medications has recently been lined out as a covariate of  
381 microbiome variation in a large-scale study (7). Due to cohort size, we had not the power to detect  
382 such effects, although they need to be assumed (Table 1).

### 383 *Clinical implementation of study results – are siblings suitable donors in FMT?*

384 It has been argued whether or not siblings or near relatives are suitable choices for donors in FMT,  
385 because they share genetic and environmental risk factors with the patients (4). A recent study on  
386 the IBD microbiome in pediatric patients and their first-grade family members suggested a disease-  
387 related microbial and metabolomic state in some relatives, suggesting this risk may be relevant (15).  
388 Moreover, Li et al. (24) recently outlined the importance of the compatibility of the donor and  
389 recipient microbiomes to receive persistent responses from FMT treatment. They concluded that  
390 each recipient will need an idiosyncratic donor to attain successful colonization by allogenic strains.  
391 Donor strains will colonize more successfully when the species is already present in the recipient  
392 microbiome. In the present study, gut microbial diversity (Shannon diversity index) and particularly  
393 species richness was significantly diminished in IBD patients when comparing each patient with  
394 their sibling. This observation of overall dysbiosis in pediatric IBD suggests that healthy siblings,  
395 who have more analogy than total strangers, but still provide a healthier microbiome composition,  
396 may indeed be suitable as donors from an efficacy point of view.

### 397 *Future outlook*

398 This study must be considered a pilot effort, by design and given the limited number of subjects  
399 included. A future study with more pediatric IBD patients and healthy siblings would yield greater  
400 statistical power and will hereby provide both further validation (esp. intra-sibling disparity) and the  
401 potential discovery of novel associations, which was very limited at the present time for gut  
402 microbial taxonomic correlates of IBD, as our research was mainly based on previous 16S rRNA  
403 findings (Appendix Table 1). Likewise, it would be very interesting to further employ direct sib pair  
404 comparisons, e.g. regarding the analyses of virulence and resistance genes. However, in the present

405 study the efficacy of direct sib pair comparison was limited for reasons of statistical power, and  
406 most analysis therefore relied on aggregated analysis (comparing the sets of all control samples to  
407 the samples in the UC/CD-cohorts), obscuring intra-sibling disparity. The additional analysis of  
408 tissue samples should be considered in the future, as recent research has demonstrated a discrepancy  
409 between luminal and mucosal samples in microbial composition (35) and as deep sequencing of  
410 bioptic samples is becoming more feasible. Today, a crucial limitation inherent to all functional  
411 metagenomic studies, excluding those using direct selection, is that only known gene families can  
412 be quantified for generation of functional profiles. Consequently, it is possible that results are  
413 reflecting research biases e.g. virulence factors to be better known in some taxa than others. With  
414 databases of curated resistance determinants growing in scope, and with technologies for large-scale  
415 functional screen metagenomics gradually maturing, these difficulties should decrease in coming  
416 years. We anticipate further studies building on top of what is reported here.

417

## 418 **Conclusion**

419 Our observations of dysbiosis, higher abundance of virulence factors and a shift towards gut  
420 bacterial taxa with known resistance gene carrying strains in the IBD population provide evidence  
421 for siblings as appropriate potential stool donors for FMT treatment of afflicted children. Pediatric  
422 IBD patients differ from their healthy siblings in several regards that can be linked to the disease.  
423 Employing such siblings as donors would further facilitate the possibility of “home-done”  
424 transplantation. Furthermore, the ethical, esthetic and clinical barriers against stool transplants  
425 likely are easier to overcome with respect to a close relative donor than in the case of an outside /  
426 adult stool donor. The same holds regarding considerations of the potential transmission of  
427 infectious diseases and the adaptations of the microflora to dietary habits, where siblings dwelling  
428 in the same home already should be similar.

429 Finally, our observations suggest utility of taking metagenomic measurements of microbial  
430 dysbiosis in both donor and recipient pre- and post-procedure in order to correlate FMT outcome  
431 with microbiome characteristics.

432 **Appendix**

433 **Appendix Table 1: Bacteria found associated with inflammatory bowel diseases by previous work groups = literature selection.**

434 ↑/↓ = increase/decrease in patients with IBD in comparison with healthy controls; ↑CD / ↓CD = increase/decrease in patients with Crohn's Disease; ↑UC / ↓UC = increase/decrease in patients with Ulcerative Colitis;  
 435 Superscripted numbers are referring to the authors, see reference table below.

Phylum	Class	Order	Family	Genus	Species
Bacteroidetes ↓ <sup>2,4,8</sup> ↑CD <sup>5</sup>	Bacteroidetes	Bacteroidales ↓CD <sup>6</sup>	Porphyromonadaceae	Odoribacter ↓ <sup>1</sup> Parabacteroides ↓CD <sup>6</sup>	
			Bacteroidaceae	Bacteroides ↑ <sup>8</sup> ↓ <sup>17</sup> ↓CD <sup>6</sup>	<i>B. thetaiotaomicron</i> ↓ <sup>2</sup> <i>B. vulgatus</i> ↓CD <sup>6</sup> <i>B. caccae</i> ↓CD <sup>6</sup>
			Prevotellaceae	Prevotella ↓UC <sup>3</sup>	
			Rikenellaceae ↓CD <sup>6</sup>	Alistipes	
Firmicutes ↓ <sup>2,4</sup> ↓UC <sup>14</sup>	Clostridia ↓UC <sup>14</sup> ↓CD <sup>3,17</sup>	Clostridiales ↓CD <sup>3,6</sup>	Lachnospiraceae ↓ <sup>1,2</sup> ↓CD <sup>6</sup>	Roseburia ↓ <sup>1</sup> ↓CD <sup>3,6</sup>	<i>R. hominis</i> ↓UC <sup>7</sup> <i>R. faecis</i> ↓CD <sup>5</sup> <i>R. intestinalis</i> ↓CD <sup>6</sup> s
				Coprococcus ↓CD <sup>6</sup>	<i>C. eutactus</i> ↓CD <sup>5</sup> <i>C. comes</i> ↓CD <sup>6</sup> s
			Clostridiaceae	Clostridium ↓ <sup>8</sup>	<i>C. leptum</i> ↓ <sup>9</sup> <i>C. nexile</i> ↓CD <sup>6</sup> s <i>C. bolteae</i> ↓CD <sup>6</sup> s
				Blautia ↓CD <sup>6</sup> s	<i>B. coccooides</i> ↓ <sup>9</sup> <i>B. hanseni</i> ↓CD <sup>6</sup>
				Dorea ↓CD <sup>6</sup>	
				Butyricoccus	<i>B. pullicaecorum</i> ↓ <sup>1</sup> ↓ <sup>16</sup>
			Ruminococcaceae ↓CD <sup>1,3,5,6</sup>	Acetivibrio <sup>1</sup>	
				Ruminococcus ↓CD <sup>1</sup> ↓CD <sup>6</sup>	<i>R. gnavus</i> ↑CD <sup>3</sup> ↓CD <sup>6</sup> <i>R. torques</i> ↓CD <sup>6</sup>
				Faecalibacterium ↓ <sup>1</sup> ↑CD <sup>10</sup> ↓CD <sup>3,6,11,17,19</sup> ↓UC <sup>12</sup>	<i>F. prausnitzii</i> ↓UC <sup>7</sup> ↓CD <sup>6</sup> ↓CD <sup>9</sup>
				Oscillospira ↓CD <sup>5,6</sup>	
				Subdoligranulum ↓CD <sup>5</sup>	
			Peptococcaceae ↓CD <sup>3</sup>	Peptococcus ↓CD <sup>3</sup>	
				Phascolactobacterium ↓ <sup>1</sup>	

Phylum	Class	Order	Family	Genus	Species
			Eubacteriaceae	Eubacterium	<i>E. rectale</i> ↓CD <sup>6</sup>
	Bacilli ↑CD <sup>3</sup>	Lactobacillales ↓ <sup>17</sup> ↑CD <sup>3</sup>	Leuconostocaceae ↓UC <sup>1</sup> ↑CD <sup>3</sup>		
			Lactobacillaceae	Lactobacillus ↑ <sup>13</sup> ↑CD <sup>3,6</sup>	
			Streptococcaceae ↓UC <sup>3</sup>	Streptococcus ↑CD <sup>6</sup> ↓UC <sup>3</sup>	
			Enterococcaceae	Enterococcus ↑CD <sup>6</sup>	
		Gemellales	Gemellaceae ↑CD <sup>6</sup>	Gemella	<i>G. morbillorum</i> ↑CD <sup>6</sup>
	Erysipelotrichi ↑UC <sup>14</sup>	Erysipelotrichiales	Erysipelotrichiaceae ↓CD <sup>6</sup> ↑CD <sup>6</sup> s	Catenibacterium ↓UC <sup>3</sup>	
	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus ↑CD <sup>3</sup>	
			Veillonellaceae ↑CD <sup>6</sup>	Veillonella ↑CD <sup>3</sup>	<i>V. parvula</i> ↑CD <sup>6</sup>
				Dialister ↓CD <sup>6</sup>	
Actinobacteria ↑ <sup>2</sup>	Actinobacteridae	Bifidobacteriales	Bifidobacteriaceae ↓CD <sup>6</sup> ↑CD <sup>6</sup>	Bifidobacterium ↓ <sup>1,11</sup> ↑ <sup>13</sup>	<i>B. bifidum</i> ↓CD <sup>6</sup> <i>B. longum</i> ↓CD <sup>6</sup> <i>B. adolescentis</i> ↓CD <sup>6</sup> <i>B. dentum</i> ↓CD <sup>6</sup> <i>B. infantis</i>
	Coriobacteridae	Coriobacteriales	Coriobacteriaceae <sup>1</sup>		
Proteobacteria ↑ <sup>2</sup> ↑UC <sup>14</sup> ↑CD <sup>3,5</sup>	Gammaproteobacteria ↑UC <sup>14</sup> ↑CD <sup>3</sup>	Enterobacteriales ↑ <sup>18</sup> ↑CD <sup>3</sup>	Enterobacteriaceae ↑CD <sup>3,4,6</sup>	Escherichia ↑ <sup>1</sup> ↑CD <sup>19</sup>	<i>E. coli</i> ↑CD <sup>6</sup> s ↑ <sup>9,11</sup> ↑UC <sup>14</sup> AIEC ↑ <sup>1</sup> ↑CD <sup>15</sup>
				Shigella ↑ <sup>1</sup> ↑CD <sup>3,19</sup>	
		Aeromonadales	Aeromonadaceae ↑CD <sup>3</sup>	Aeromonas ↑CD <sup>3</sup>	
		Pasteurellales	Pasteurellaceae ↑CD <sup>6</sup>	Haemophilus (spp.) ↑ CD <sup>6</sup>	<i>H. parainfluenzae</i> ↑CD <sup>6</sup>
	Betaproteobacteria	Neisseriales	Neisseriaceae ↑CD <sup>6</sup>	Eikenella	<i>E. corrodens</i> ↑CD <sup>6</sup>
		Burkholderiales	Sutterellaceae	Sutterella ↓CD <sup>6</sup>	
	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Bilophila ↓CD <sup>6</sup>	<i>B. wadsworthia</i> ↓ <sup>20</sup>
				Desulfovibrio	
	Alphaproteobacteria	Rhizobiales ↑CD <sup>5</sup>	Bradyrhizobiaceae ↑CD <sup>5</sup>		
Fusobacteria ↑CD <sup>3</sup> ↑UC <sup>14</sup>	Fusobacteria ↑CD <sup>3</sup>	Fusobacteriales ↑CD <sup>3</sup>	Fusobacteriaceae ↑CD <sup>3,6</sup> ↓CD <sup>6</sup>	Fusobacterium ↑CD <sup>3</sup>	<i>F. nucleatum</i> ↑CD <sup>6</sup>
Spirochaetae	Spirochaetes ↑UC <sup>14</sup>	Spirochaetales			
Verrucomicrobia ↓UC <sup>14,18</sup>	Verrucomicrobiae ↓UC <sup>18</sup>	Verrucomicrobiales ↓UC <sup>18</sup>	Verrucomicrobiaceae ↓UC <sup>18</sup>		
Lentisphaerae ↓UC <sup>14</sup>					
Tenericutes ↓ <sup>3</sup>	Mollicutes ↓ <sup>3</sup>	Anaeroplasmatales ↓ <sup>3</sup>	Anaeroplasmataceae ↓ <sup>3</sup>	Asteroleplasma ↓ <sup>3</sup>	

Ref-Nr.	Author	Methods	Study cohort
1	Morgan et al., 2012	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> <li>• <b>WGS</b> of 11 fecal samples</li> </ul>	<ul style="list-style-type: none"> <li>• 121 CD</li> <li>• 75 UC</li> <li>• 27 Controls</li> <li>• Age: 13-45 y</li> </ul>
2	Frank et al., 2007	<ul style="list-style-type: none"> <li>• Q-PCR -rRNA sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 190 CD, UC, Controls</li> </ul>
3	Willing et al., 2010	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 29 CD</li> <li>• 16 UC</li> <li>• 35 Controls</li> <li>• Age: 40-67 y</li> </ul>
4	Walker et al., 2011	<ul style="list-style-type: none"> <li>• 16S rRNA- sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 6 CD</li> <li>• 6 UC</li> <li>• 5 Controls</li> <li>• Age: 24-73 y</li> </ul>
5	Kaakoush et al., 2012	<ul style="list-style-type: none"> <li>• 16S rRNA- sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 19 CD (n.o.)</li> <li>• 21 Controls</li> <li>• Age: 5-15 y</li> </ul>
6	Gevers et al., 2014	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> <li>• <b>WGS</b> of 43 Fecal samples</li> </ul>	<ul style="list-style-type: none"> <li>• 447 CD (n.o.)</li> <li>• 221 Controls</li> <li>• Age: 3-17 y</li> </ul>
7	Machiels et al., 2013	<ul style="list-style-type: none"> <li>• 16S rRNA sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 127 UC</li> <li>• 87 Controls</li> </ul>
8	Andoh et al., 2011	<ul style="list-style-type: none"> <li>• 16S rRNA sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 31CD</li> <li>• 31 UC</li> <li>• 30 Controls</li> </ul>
9	Duboc et al., 2013	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 12 CD</li> <li>• 30 UC</li> <li>• 29 Controls</li> <li>• Age: 20-60 y</li> </ul>
10	Hansen et al., 2012	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 13 CD</li> <li>• 12 UC</li> <li>• 12 Controls</li> <li>• Age: Children</li> </ul>
11	Schwartz et al., 2010	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 69 IBD</li> <li>• 25 Controls</li> <li>• Age: 1-20</li> </ul>
12	Varela et al., 2013	<ul style="list-style-type: none"> <li>• qRT-PCR</li> </ul>	<ul style="list-style-type: none"> <li>• 116 UC patients</li> <li>• 29 first degree relatives</li> <li>• 31 Controls</li> <li>• Age 18-75 y</li> </ul>
13	Wang et al., 2014	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 36 CD</li> <li>• 63 UC</li> <li>• 21 Controls</li> </ul>
14	Michail et al., 2012	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 27 UC</li> <li>• 26 Controls</li> <li>• Mean Age: 13,5 y</li> </ul>
15	Martinez-Medina et al., 2009	<ul style="list-style-type: none"> <li>• Colony dependent Rep-PCR</li> <li>• Pulsed field gel electrophoresis</li> <li>• Adhesion and invasion assays</li> </ul>	<ul style="list-style-type: none"> <li>• 20 CD</li> <li>• 28 Controls</li> </ul>
16	Eeckhaut et al., 2013	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 51 CD</li> <li>• 91 UC</li> <li>• 88 Controls</li> <li>• Mean Age: 40 y</li> </ul>
17	Aomatsu et al., 2012	<ul style="list-style-type: none"> <li>• 16S rRNA gene sequencing</li> <li>• T-RFLP- analysis</li> </ul>	<ul style="list-style-type: none"> <li>• 10 CD</li> <li>• 14 UC</li> <li>• 27 Controls</li> </ul>

Ref-Nr.	Author	Methods	Study cohort
			<ul style="list-style-type: none"> <li>• Age: 1-18 y</li> </ul>
18	Papa et al., 2012	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 23 CD</li> <li>• 43 UC</li> <li>• 24 Controls</li> <li>• Mean Age: 13 y</li> </ul>
19	Thorkildsen et al., 2013	<ul style="list-style-type: none"> <li>• 16S rRNA-sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• 30 CD</li> <li>• 33 UC</li> <li>• 33 Controls</li> <li>• Mean Age: 33 y</li> </ul>
20	Jia W et al., 2012	<ul style="list-style-type: none"> <li>• PCR of the dsrAB gene</li> </ul>	<ul style="list-style-type: none"> <li>• 20 CD</li> <li>• 14 UC</li> <li>• 18 Controls</li> </ul>

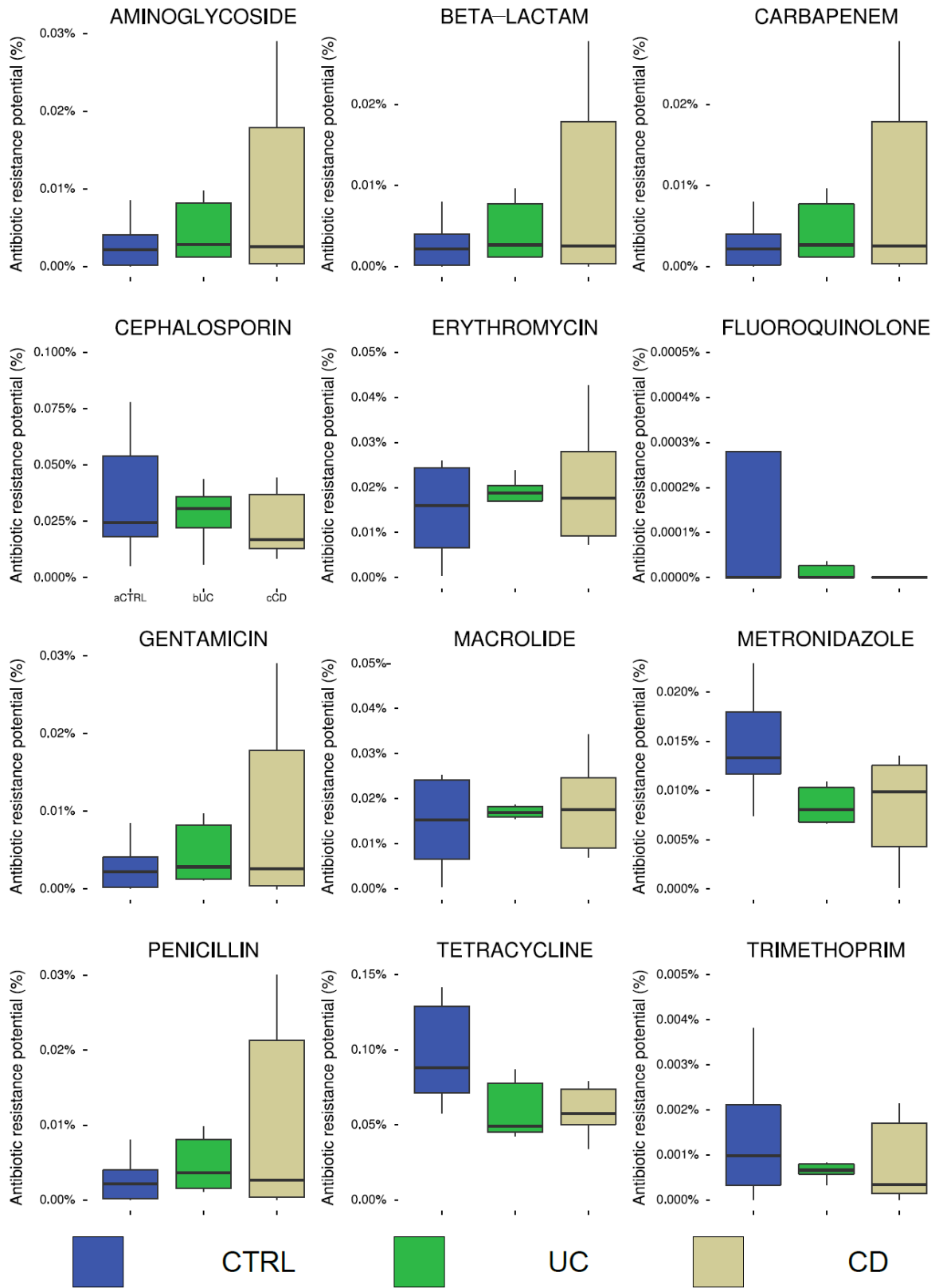
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Appendix Figure 1: Antibiotic resistance potential for the different classes of antibiotics which have been used by the study population. The antibiotic resistance potential is shown as box plots. No significant difference in antibiotic resistance potential was observed.

494 **Acknowledgements**

495 This publication comprises results from R. L. Knoll's medical doctor thesis.

496 **Grants**

497 This work was supported by the "Naturwissenschaftlich-medizinsches Forschungszentrum (NMFZ) ",  
498 University Medical Centre of the Johannes Gutenberg University, Mainz, Germany; and FP7: MetaCardis,  
499 grant agreement [HEALTH-2012-305312]. The funding bodies had no direct role in the design or conduct of  
500 the study, collection, management, analysis or interpretation of the data, or preparation, review or approval of  
501 the manuscripts.

502 **Disclosures**

503 No conflicts of interest, financial or otherwise, are declared by the authors.

504 **Author Contributions**

505 R.L.K.: Study concept and design; collection, analysis and interpretation of data; drafting of the  
506 manuscript. K.F.: Study concept and design, analysis and interpretation of data; drafting of the  
507 manuscript; critical revision of the manuscript for important intellectual content. J.R.K. and S.S.:  
508 analysis of data; critical revision of the manuscript for important intellectual content. U.K.: Study  
509 design; acquisition of data, critical revision of the manuscript for important intellectual content.  
510 C.U.M.: contributed to the design of the study, critical revision of the manuscript for important  
511 intellectual content. P.B.: Study concept and design; critical revision of the manuscript; obtained  
512 funding; S.G.: Study concept and design; acquisition of data; critical revision of the manuscript for  
513 important intellectual content; obtained funding. All authors approved the final version of the  
514 manuscript.

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660 **Figure Captions**

661 **Figure 1: Shannon diversity index, species richness and evenness in comparison between groups (CTRL,**  
662 ***UC, CD).*** Boxplots show all samples contrasted with MWU tests, showing significances as +:  $FDR < 0.1$ , \*:  $FDR < 0.05$ . Scatterplots show each sib pair, revealing significantly higher diversity of each type in controls  
664 *than in siblings with IBD.*

665 **Figure 2:**

666 ***A) Taxonomic distribution of bacterial taxa in relation to their relative abundance compared between the***  
667 ***control cohort and both IBD populations (UC = Ulcerative colitis; CD = Crohn's disease).*** Nodes represent  
668 *taxa. Node color indicates direction of associations, with white nodes marking cases where an association*  
669 *from Appendix Table 1 could not be validated in the novel dataset (see bottom of figure for colour key).*  
670 *Coloured nodes with a narrow edge represent associations from the literature set (Appendix Table 1) which*  
671 *approached significance (MWU  $FDR < 0.1$ ) when testing only hypotheses from this set. Coloured nodes with*  
672 *a bold edge represent such associations which also approached significance (MWU or KW  $FDR < 0.1$ )*  
673 *without restricting the hypothesis space. Coloured nodes with a bold, dashed outer edge represent IBD*  
674 *associations approaching significance (MWU or KW  $FDR < 0.1$ ) when testing all possible associations,*  
675 *which are novel relative to the set of previous literature-derived findings. Detailed FDR scores and results*  
676 *from sibpair testing are given in Supplementary Table 1. Abbreviations: E. rectale = Eubacterium rectale, R.*  
677 *gnavus = Ruminococcus gnavus, F. prausnitzii = Faecalibacterium prausnitzii, C. ramosum = Clostridium*  
678 *ramosum, F. nucleatum = Fusobacterium nucleatum, E. coli = Escherichia coli, B. wadsworthia = Bilophila*  
679 *wadsworthia.*

680 ***B) Box plots of relative abundance of species in % (square root scale for visibility) for which differences in***  
681 ***abundance approached significance (\* indicates MWU  $FDR < 0.1$ ) in this cohort, comparing the sets of***  
682 ***UC or CD samples to the set of Controls.*** Detailed FDR scores and results from sibpair testing are given in  
683 *Supplementary Table 1. The observed species-level increase of C. ramosum in UC compared to controls is*  
684 *novel relative to the set of literature-derived findings.*

685 **Figure 3: Correlation of species abundance with the abundance of virulence genes significantly increased**  
686 ***in the microbiomes of UC patients.*** Dark colored = positive correlation of species abundance and the  
687 *abundance of the corresponding virulence gene ( $SCC > 0.65$ ,  $FDR < 0.1$ ), red = increase of the species*

688 *abundance in UC patients (MWU, FDR < 0.1), blue = no significant difference of abundance of species*  
689 *between UC and Control.*

690 **Figure 4: Mean lifetime number of antibiotic courses in comparison between Control, Ulcerative colitis**  
691 **(UC) and Crohn's disease (CD).** *Bar charts are divided into segments representing the different antibiotic*  
692 *classes covered. For the most frequently used antibiotics, mean lifetime number of therapies are indicated.*

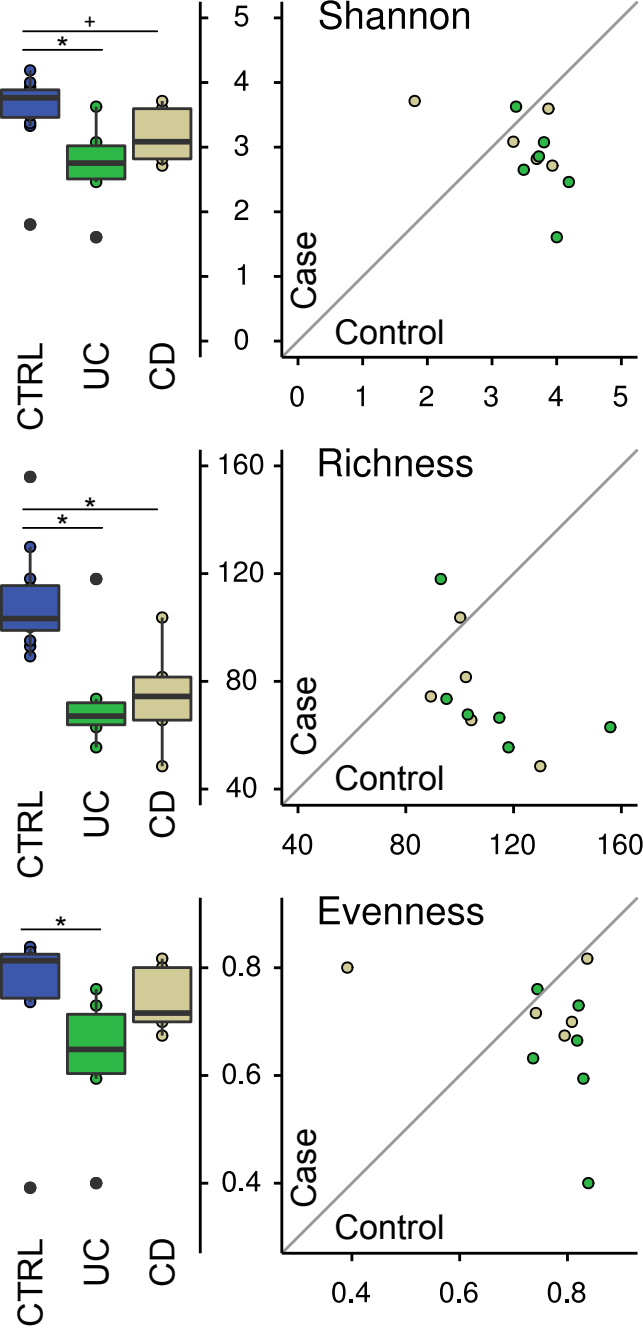
693 **Figure 5: Fraction of potential carrier species:** *For relative abundance of potentially resistant species,*  
694 *significant difference was observed for each antibiotic class between control and Ulcerative colitis (UC) (BY*  
695 *FDR = 0.062) and control and Crohn's disease (BY FDR = 0.019).*

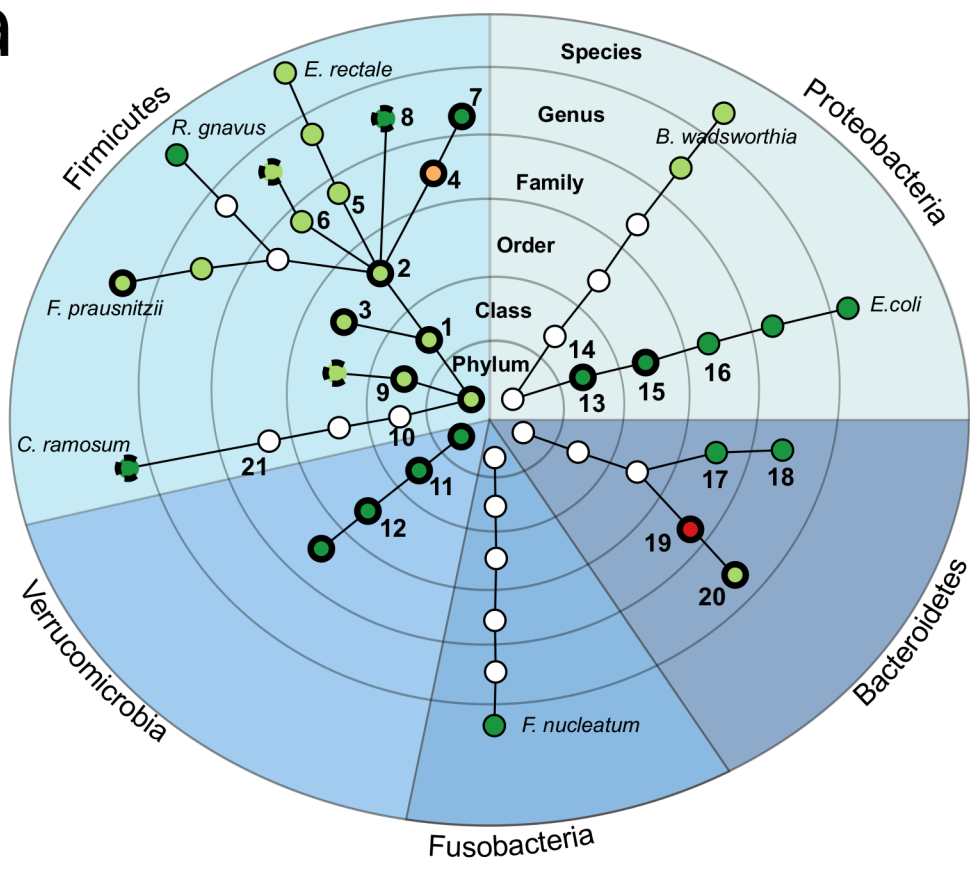


696 Table 1: Cohort characteristics

Characteristics	UC	CD	Control
n =	6	6	12
Demographics			
male: female	2:4	3:3	6:6
Age (years)			
Median ± SD	13 ± 2.7	14 ± 2.0	12.5 ± 3.6
Range	10 - 17	11 - 16	8 - 20
BMI (z-score)			
Median ± SD	-0.09 ± 0.73	-0.10 ± 1.09	0.26 ± 1.10
< -1 (%)	0	34	25
> +1 (%)	17	0	34
Age at onset (years)			
Median ± SD	9.5 ± 2.8	10 ± 1.8	
Range	4 - 12	9 - 14	
Disease duration (months)			
Median ± SD	22.5 ± 54.4	29 ± 31.6	
Range	13 - 144	0 - 82	
Disease activity			
inactive	3	1	
mild	1	2	
moderate-severe	2	3	
Medications (%)			
Steroids	67	50	
Anti-TNF	17	50	
Azathioprine	50	34	
Mesalazine	50	17	
TGF-β2	0	34	
UDCA	34	0	
Colchizine	17	0	

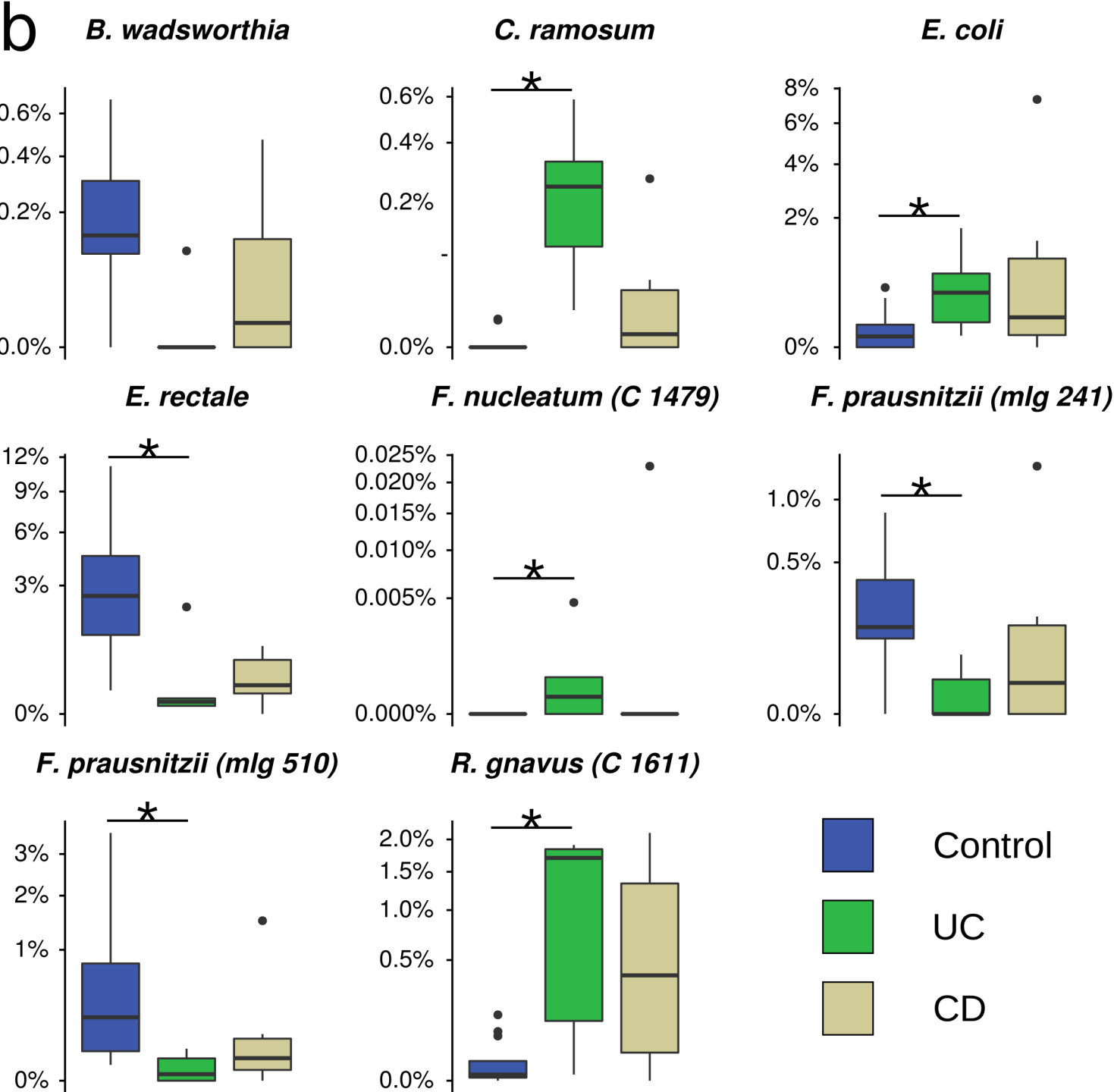
697 UC = Ulcerative colitis, CD = Crohn's disease; % = per group (UC, CD, Control); Disease activity based on PUCAI,  
698 PCDAI, respectively; Anti-TNF = infliximab/adalimumab; TGF-β2 = transforming growth factor beta 2 nutritional support  
699 formula; UDCA = ursodeoxycholic acid

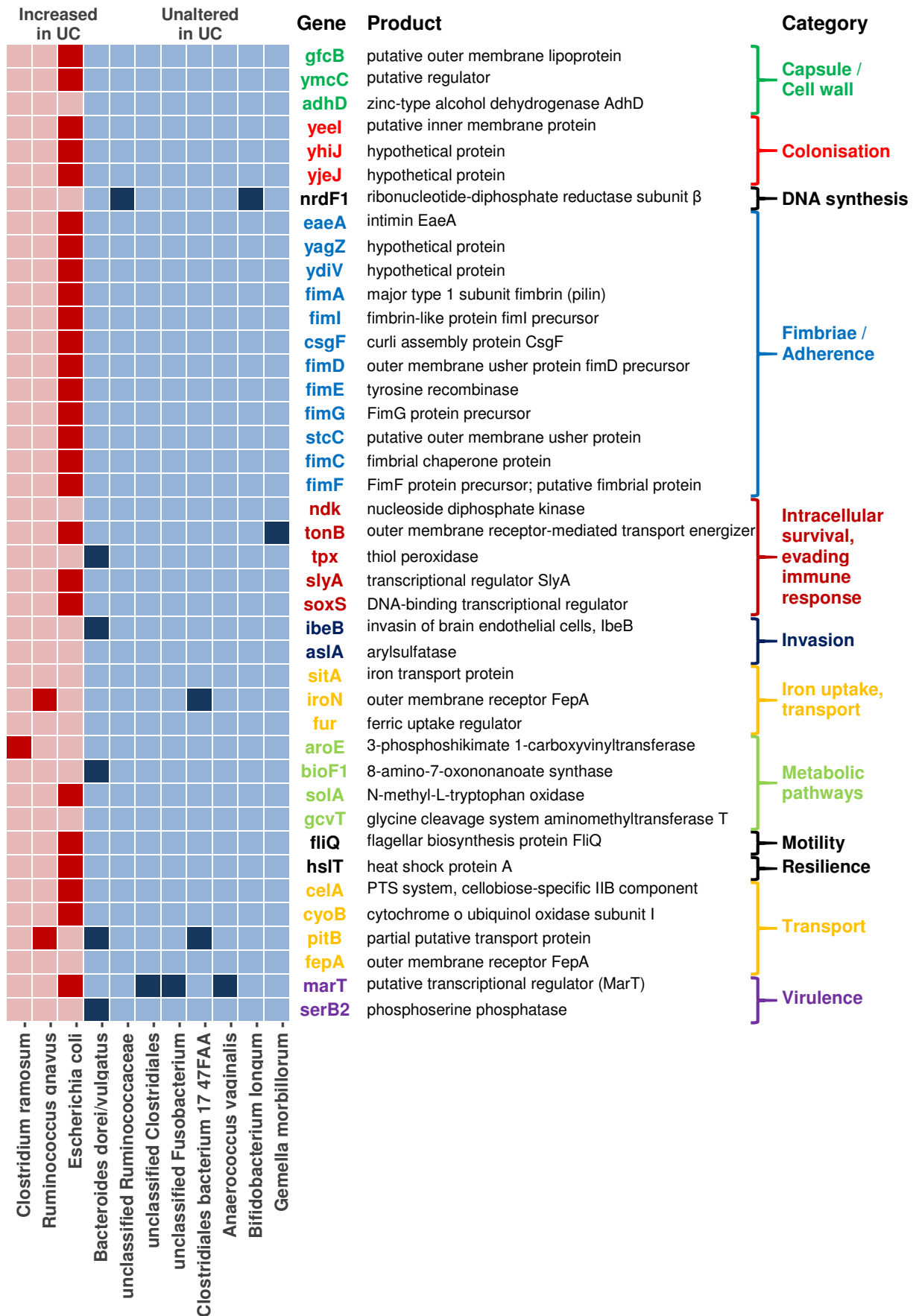


**a**

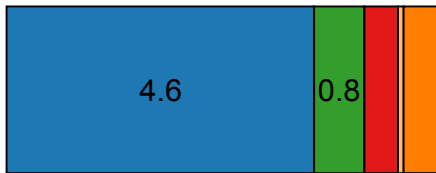
- 1 *Clostridia*
- 2 *Clostridiales*
- 3 unknown *Clostridia*
- 4 *Clostridiaceae*
- 5 *Eubacteriaceae*
- 6 unknown *Clostridiales*
- 7 *Clostridium (Clostridiaceae)*
- 8 *Anaerococcus*
- 9 unknown *Firmicutes*
- 10 *Erysipelotrichi*
- 11 *Verrucomicrobiae*
- 12 *Verrucomicrobiales*
- 13 *Gammaproteobacteria*
- 14 *Deltaproteobacteria*
- 15 *Enterobacteriales*
- 16 *Enterobacteriaceae*
- 17 *Bacteroidaceae*
- 18 *Bacteroides*
- 19 *Porphyromonadaceae*
- 20 *Parabacteroides*
- 21 *Erysipelotrichiaceae*

- Literature set finding, validated
- Independent rediscovery
- Enriched in CD
- Enriched in UC
- Literature set finding, not validated
- Novel association
- Enriched in CD & UC
- Depleted in UC

**b**



CTRL



UC



CD



- AMINOGLYCOSIDE
- BETA-LACTAM
- FLUOROQUINOLONE
- MACROLIDE
- METRONIDAZOLE
- SULPHONAMIDE
- TETRACYCLINE
- TRIMETHOPRIM

0.0 2.5 5.0 7.5 10.0 12.5

Average number of lifetime doses

