

1 **Gut microbiota composition and *Clostridium difficile* infection in hospitalized elderly**  
2 **individuals: a metagenomic study**

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9 **Supplementary information**

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## 1 **Supplementary figures legends**

2 **Figure S1:** Taxonomic profiles of the 84 analyzed 16S rRNA profiling datasets. The bar plot  
3 shows taxonomy profiles at genera level of taxa with relative abundance >1 %. AB-, AB+  
4 and CDI datasets are grouped separately. (CDI: *Clostridium difficile* infected samples; AB+:  
5 *Clostridium difficile*-negative samples, patients exposed to antibiotic treatment; AB-:  
6 *Clostridium difficile*-negative samples, patients not exposed to antibiotic treatment).

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8 **Figure S2.** Functional changes in the gut microbiome of AB+ and CDI compared to AB-  
9 subjects, part 1. Panel a shows relative abundance of *Clostridium difficile* microbial  
10 biomarkers (CDI<sub>mb</sub>) in AB-, AB+ and CDI shotgun metagenomic datasets. Panel b reports  
11 the relative abundance of taxa with supposed health-promoting activities and opportunistic  
12 pathogens in AB-, AB+ and CDI samples. Panel c illustrates percentage variation in  
13 abundance of enzymes and transporters involved in antibiotic resistance in AB+ and CDI  
14 compared to AB- subjects as well as CDI compared to AB+ subjects. Panel d depicts  
15 variations of abundance of Cluster of Orthologous Genes (COG) functional families involved  
16 in energy production in AB+ and CDI compared to AB- samples. COG functional families  
17 are abbreviated as follows: [C] Energy production and conversion, [G] Carbohydrate  
18 transport and metabolism, [E] Amino acid transport and metabolism. Panel e shows changes  
19 in Glycosyl Hydrolase (GH) families involved in glucose (circled in green) and fructose  
20 (circled in purple) production in AB+ and CDI compared to AB- datasets. Panel f exhibits  
21 variation in bile salts and bile acid degradation pathways in AB+ and CDI compared to AB-  
22 samples as well as CDI compared to AB+ subjects. Panel g displays variation of pathways  
23 involved in butyrate production in AB+ and CDI compared to AB- samples as well as CDI  
24 compared to AB+ subjects. Panel h shows variation of pathways involved in succinate  
25 production in AB+ and CDI compared to AB- samples as well as CDI compared to AB+

1 subjects. (CDI: *Clostridium difficile* infected samples; AB+: *Clostridium difficile*-negative  
2 samples, patients exposed to antibiotic treatment; AB-: *Clostridium difficile*-negative  
3 samples, patients not exposed to antibiotic treatment).

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5 **Figure S3.** Profiling of *Peptostreptococcaceae* population based on shotgun metagenomics  
6 data in AB-, AB+ and CDI samples. Panel a shows cake diagrams depicting the taxonomic  
7 composition at species level of the AB-, AB+ and CDI datasets. Panel b displays bar plots  
8 presenting the variation in relative abundance of the mapped *Peptostreptococcaceae* species  
9 in the AB-, AB+ and CDI subjects. Panel c illustrates, by means of bar plots, the variation in  
10 the commensals/(opportunistic) pathogens ratio observed for the *Peptostreptococcaceae*  
11 family population in AB-, AB+ and CDI datasets. (CDI: *Clostridium difficile* infected  
12 samples; AB+: *Clostridium difficile*-negative samples, patients exposed to antibiotic  
13 treatment; AB-: *Clostridium difficile*-negative samples, patients not exposed to antibiotic  
14 treatment)

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**Table S1.** Filtering report of 16S rRNA profiling datasets.

Sample	Number of reads	Number of reads removed because of:					Reverse primers not found	Final reads number	average reads length
		Outside bounds (100-400)	Ambiguous bases	Mean quality <25	Homopolymer runs >7bp	Primer mismatch >1			
1AB-	120015	25611	0	15844	296	23462	13018	41784	187.4
2AB-	150273	34468	0	15725	648	14499	28779	56153	182.7
3AB-	90574	15984	0	7645	267	11012	9389	46275	188.9
4AB-	91739	22463	0	10853	787	6346	17404	33882	181.4
5AB-	160563	35110	0	16634	36	15115	17926	75742	188.3
6AB-	77214	10825	0	8957	131	9232	10356	37713	192.1
7AB-	86507	10776	0	11486	173	16364	16829	30878	188.8
8AB-	97352	28937	0	9087	160	6649	14244	38273	182.5
9AB-	162000	45484	0	18677	639	13246	23667	60287	181.7
10AB-	123875	25812	0	11418	893	10878	15651	59223	183.1
11AB-	114148	28437	0	16763	131	7335	18555	42927	175.6
12AB-	147650	60398	0	9197	1490	10233	18371	47961	169.2
13AB-	194079	64295	0	58643	87	7484	13898	49672	170.2
14AB-	178628	65987	0	21501	83	16174	20809	54074	157.8
15AB-	122748	38119	0	17612	272	7774	15756	43215	176.3
16AB-	89617	21741	0	9565	158	9297	8347	40509	189.8
17AB-	124480	35139	0	10292	65	8725	24926	45333	170.2
18AB-	129985	36348	0	8056	43	9998	27675	47865	168.4
19AB-	212917	61536	0	18144	670	10070	27260	95237	170.6
20AB-	90294	30311	0	7886	310	4862	14675	32250	167.9
21AB-	147459	49699	0	9530	111	13506	20887	53726	171
22AB-	112246	42991	0	10420	819	6148	17191	34677	167
23AB-	87547	18622	0	4511	250	5299	7610	51255	173
24AB-	132896	46046	0	9815	484	11019	16466	49066	172.5
25AB-	167416	48641	0	15847	298	15386	26977	60267	168.5
26AB-	145410	34350	0	17439	136	11172	32881	49423	181.9
27AB-	173005	48307	0	11260	749	9762	17432	85495	186.7
28AB-	77540	11782	0	7957	189	4520	11743	41349	190.3
29AB-	118190	24809	0	11886	157	9233	14895	57210	191.4
30AB-	92259	20246	0	8127	150	8940	14568	40228	190
1AB+	124659	23180	0	16251	176	10894	20302	53856	174.6
2AB+	82771	19963	0	13018	119	6166	24534	18970	174.2
3AB+	216471	83802	0	13894	382	21343	33787	63263	170.9
4AB+	126768	47446	0	22817	179	7681	10611	38034	167.9
5AB+	138992	39084	0	21111	95	12274	22720	43708	172.3
6AB+	119878	19465	0	13670	194	11898	39634	35017	186
7AB+	184040	55783	0	48059	24	10458	24976	44740	169.4
8AB+	73703	19219	0	4568	437	4642	13249	31588	168.4
9AB+	105604	26873	0	6429	191	6331	20966	44813	168.2
10AB+	99644	19952	0	10856	131	8722	6668	53315	177.1
11AB+	114953	30041	0	11576	60	7386	12726	53156	178.8
12AB+	95181	20791	0	8336	121	7714	6249	51970	180.3
13AB+	58793	25459	0	3728	77	3001	5254	21274	170.8

14AB+	192800	68806	0	12214	1236	11039	26415	73090	167.6
15AB+	155536	45816	0	40475	141	7015	32043	30046	163.5
16AB+	121227	52190	0	7261	1190	3747	14700	42139	165.3
17AB+	162464	65499	0	11420	337	11104	19654	54450	169.7
18AB+	148437	46913	0	8493	405	10080	22871	59675	169.7
19AB+	96451	41524	0	4608	237	5422	11571	33089	168.9
20AB+	193373	42968	0	10981	275	15926	18517	104706	179.4
21AB+	125361	28892	0	6566	287	9754	14351	65511	178.4
22AB+	129020	38532	0	8335	262	9598	20093	52200	172.8
23AB+	116035	39367	0	12692	238	6669	22268	34801	169.7
24AB+	170079	59554	0	15168	1210	11587	23097	59463	167.8
25AB+	139482	54150	0	10116	183	11330	23721	39982	167.5
26AB+	152175	54927	0	16274	194	5879	27817	47084	165.3
27AB+	56425	8284	0	5769	39	6446	13751	22136	188.8
28AB+	146431	49403	0	11290	243	10922	30965	43608	170.4
29AB+	87228	17121	0	8685	163	8697	11812	40750	187.5
1CDI	146627	27395	0	17164	41	21820	28161	52046	182.6
2CDI	158053	32477	0	12869	36	15843	11433	85395	180
3CDI	108358	22376	0	13727	75	9639	30481	32060	167
4CDI	91899	28451	0	11783	49	9095	15969	26552	175.4
5CDI	152213	22158	0	17148	107	20023	53099	39678	165.7
6CDI	115112	19433	0	34021	44	9265	35326	17023	157.1
7CDI	182926	36499	0	25893	60	14135	47332	59007	174.8
8CDI	145673	28459	0	11771	135	15740	38029	51539	174.3
9CDI	101190	25146	0	8822	167	5687	17050	44318	173.2
10CDI	171412	24157	0	11967	131	24002	25385	85770	174.3
11CDI	128997	38193	0	8355	191	13205	18988	50065	171.5
12CDI	84573	23951	0	15418	205	3300	12209	29490	173.5
13CDI	132382	38732	0	13046	256	9706	34317	36325	172.6
14CDI	123579	29681	0	14228	111	10285	41162	28112	169
15CDI	184050	58818	0	10763	493	15846	24403	73727	177.1
16CDI	146186	16720	0	17018	127	21730	36819	53772	186.9
17CDI	112366	34677	0	27145	38	7643	25841	17022	167
18CDI	138705	21590	0	15177	138	17018	23796	60980	187.8
19CDI	111768	29306	0	10762	87	10982	23976	36655	185.9
20CDI	11960	0	0	0	1	236	6	11717	191.6
21CDI	31429	0	0	1	1	843	8	30576	193.2
22CDI	34375	0	0	0	4	1045	9	33317	190.8
23CDI	33355	0	0	1	2	837	9	32506	190.5
24CDI	19737	0	0	0	4	348	9	19376	188.6
25CDI	39032	0	0	0	1	727	14	38290	194.5

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**Table S2.** Variations of taxa (> 0.1 %) between AB-, AB+ and CDI averages.

Taxa <sup>o*</sup>	AB-	AB+	CDI	AB+ vs AB-	P-value*	CDI vs AB+	P-value*	CDI vs AB-	P-value*
<u>U. m. of Blastocystis order</u>	0.10%	0.01%	0.00%	-87.30%	0.21	-100.00%	0.18	-100.00%	0.23
<u>U. m. of CFT112H7 family</u>	0.95%	0.47%	0.00%	-50.55%	0.39	-99.90%	0.41	-99.95%	0.13
<u>Fonticella</u>	0.19%	0.00%	0.00%	-100.00%	0.55	-	0.13	-99.73%	0.3
<u>Dialister</u>	0.54%	1.95%	0.01%	257.20%	0.76	-99.73%	0.47	-99.02%	0.11
<u>Megasphaera</u>	0.14%	0.22%	0.00%	55.35%	0.61	-99.37%	0.28	-99.02%	0.19
<u>Thalassospira</u>	0.39%	0.12%	0.00%	-69.49%	0.23	-96.66%	0.7	-98.98%	0.17
<u>U. m. of Comamonadaceae family</u>	0.73%	0.01%	0.01%	-98.18%	0.52	-37.66%	0.61	-98.87%	0.3
<u>Pyramidobacter</u>	1.17%	0.16%	0.02%	-86.08%	0.27	-90.58%	0.39	-98.69%	0.3
<u>Parvimonas</u>	0.19%	0.00%	0.00%	-99.51%	0.15	174.62%	0.22	-98.67%	0.59
<u>Cloacibacillus</u>	0.29%	0.34%	0.00%	17.19%	0.95	-98.64%	0.29	-98.41%	0.28
<u>Salinibacter</u>	0.14%	0.00%	0.00%	-100.00%	0.75	-	0.01	-97.24%	0.47
<u>Anaerotruncus</u>	0.87%	0.43%	0.03%	-50.20%	0.07	-92.79%	0.06	-96.41%	0.03
<b>U. m. of Christensenellaceae family</b>	1.13%	2.08%	0.06%	83.59%	0.48	-97.20%	0.06	-94.86%	0.006
U. m. of Porphyromonadaceae family	0.42%	0.27%	0.02%	-36.72%	0.76	-91.29%	0.03	-94.49%	0.09
U. m. of Bacteroidetes phylum	0.63%	0.62%	0.04%	-2.67%	0.92	-93.73%	0.19	-93.90%	0.27
<i>RC9 gut group</i>	0.68%	0.58%	0.04%	-14.64%	0.87	-92.83%	0.28	-93.88%	0.25
U. m. of Bacteria kingdom	1.02%	2.12%	0.06%	107.36%	0.4	-96.98%	0.2	-93.74%	0.11
<i>Odoribacter</i>	0.43%	0.96%	0.03%	120.85%	0.33	-96.99%	0.06	-93.35%	0.09
<i>Butyrivibrio</i>	0.35%	0.19%	0.02%	-46.55%	0.62	-87.51%	0.19	-93.32%	0.29
<i>Barnesiella</i>	1.27%	1.03%	0.11%	-18.73%	0.68	-88.97%	0.02	-91.04%	0.01
<i>Butyricimonas</i>	0.40%	0.48%	0.04%	21.01%	0.95	-92.34%	0.02	-90.73%	0.001
<i>Collinsella</i>	0.39%	0.26%	0.04%	-33.80%	0.4	-85.93%	0.006	-90.68%	0.03
<u>Lachnospira</u>	3.28%	1.22%	0.31%	-62.84%	0.23	-74.81%	0.19	-90.64%	0.005
<u>Alistipes</u>	10.73%	4.61%	1.25%	-57.07%	0.001	-72.87%	0.06	-88.35%	0.0002
<i>Acidaminococcus</i>	0.04%	0.12%	0.01%	209.92%	0.42	-95.69%	0.14	-86.64%	0.34
<i>Ruminococcus</i>	0.98%	1.51%	0.13%	54.71%	0.28	-91.10%	0.06	-86.24%	0.17
<b>U. m. of Ruminococcaceae family</b>	11.20%	9.76%	1.82%	-12.81%	0.29	-81.39%	0.002	-83.77%	<.0001
U. m. of Family XIII family	0.10%	0.16%	0.02%	66.28%	0.52	-89.70%	0.32	-82.87%	0.22
U. m. of Clostridia class	0.05%	0.17%	0.01%	257.36%	0.64	-94.63%	0.42	-80.82%	0.17
<i>Oscillospira</i>	0.22%	0.38%	0.05%	67.97%	0.1	-88.02%	0.0004	-79.88%	0.0002
U. m. of vadinBB60 family	0.40%	0.67%	0.08%	66.51%	0.78	-87.52%	0.31	-79.23%	0.18
U. m. of Defluviitaleaceae family	0.25%	0.57%	0.06%	123.71%	0.2	-89.98%	0.28	-77.58%	0.4
U. m. of NB1-n order	0.00%	0.11%	0.00%	7622.71%	0.12	-99.69%	0.14	-76.44%	0.07
<i>Subdoligranulum</i>	0.45%	1.19%	0.11%	161.37%	0.21	-90.33%	0.04	-74.72%	0.03
<u>U. m. of Prevotellaceae family</u>	1.82%	0.82%	0.54%	-54.64%	0.53	-34.85%	0.77	-70.45%	0.27
<b>U. m. of Clostridiales order</b>	0.76%	1.32%	0.25%	74.93%	0.13	-81.47%	0.008	-67.59%	0.001
<i>Faecalibacterium</i>	1.49%	2.20%	0.50%	47.78%	0.34	-77.49%	0.11	-66.74%	0.16
<b>U. m. of Bacteroidales order</b>	0.28%	0.24%	0.10%	-12.77%	0.3	-59.83%	0.07	-64.96%	0.02
<i>Bacteroides</i>	29.59%	31.33%	11.86%	5.89%	0.61	-62.15%	0.002	-59.92%	0.004
<b>U. m. of Lachnospiraceae family</b>	13.64%	11.81%	6.12%	-13.37%	0.74	-48.20%	0.09	-55.12%	0.04
<i>Bifidobacterium</i>	0.65%	1.23%	0.32%	90.55%	0.49	-74.01%	0.14	-50.48%	0.2

<i>Alloprevotella</i>	0.03%	0.18%	0.02%	583.16%	0.46	-90.95%	0.25	-38.19%	0.76
<i>Desulfovibrio</i>	0.16%	0.25%	0.11%	55.23%	0.45	-58.12%	0.34	-34.99%	0.63
<i>Parabacteroides</i>	1.87%	2.73%	1.24%	46.08%	0.25	-54.65%	0.31	-33.76%	0.34
<i>Phascolarctobacterium</i>	0.38%	0.67%	0.25%	77.57%	0.09	-62.11%	0.18	-32.71%	0.43
<i>Streptococcus</i>	0.30%	0.20%	0.20%	-31.67%	0.85	-0.74%	0.83	-32.18%	0.64
<i>Bilophila</i>	0.12%	0.04%	0.09%	-63.75%	0.02	105.74%	0.56	-25.42%	0.67
<i>Intestinimonas</i>	0.37%	0.60%	0.27%	65.24%	0.99	-54.62%	0.42	-25.01%	0.28
U. m. of S24-7 family	0.60%	0.19%	0.49%	-68.62%	0.08	162.98%	0.15	-17.48%	0.77
U. m. of Firmicutes phylum	0.11%	1.37%	0.09%	1158.62%	0.35	-93.32%	0.41	-15.86%	0.91
<i>Lactobacillus</i>	0.72%	0.47%	0.68%	-34.81%	0.88	44.51%	0.81	-5.80%	0.59
<i>Blautia</i>	0.60%	0.85%	0.58%	42.71%	0.15	-31.39%	0.49	-2.08%	0.69
U. m. of Erysipelotrichaceae family	0.06%	0.56%	0.07%	797.00%	0.16	-87.69%	0.28	10.40%	0.63
<i>Prevotella</i>	1.82%	1.68%	2.66%	-7.55%	0.78	58.27%	0.84	46.32%	0.51
U. m. of Clostridiaceae 1 family	0.11%	0.15%	0.20%	35.45%	0.93	35.25%	0.82	83.20%	0.48
<i>Paraprevotella</i>	0.10%	0.39%	0.24%	286.35%	0.25	-37.95%	0.76	139.73%	0.48
<i>Succinivibrio</i>	0.00%	0.61%	0.00%	109131.67%	0.8	-99.75%	0.21	169.83%	0.11
<i>Akkermansia</i>	0.60%	1.88%	3.60%	213.03%	0.08	91.68%	0.18	500.02%	0.16
<i>Sutterella</i>	0.52%	0.51%	4.98%	-2.77%	0.65	878.81%	0.19	851.74%	0.15
<i>Coprobacter</i>	0.14%	0.17%	1.29%	22.29%	0.35	680.83%	0.41	854.86%	0.38
<i>Escherichia-Shigella</i>	0.92%	0.89%	10.69%	-3.47%	0.6	1105.91%	0.55	1064.02%	0.006
<i>Catenibacterium</i>	0.01%	0.01%	0.16%	-33.58%	0.57	2513.07%	0.15	1635.63%	0.25
U. m. of Peptostreptococcaceae family	0.12%	0.32%	2.58%	175.28%	0.25	701.23%	0.08	2105.62%	0.13
<i>Morganella</i>	0.01%	0.00%	0.32%	-97.13%	0.19	78189.14%	0.2	2148.48%	0.24
U. m. of Lactobacillales order	0.01%	0.05%	0.25%	394.86%	0.07	361.84%	0.07	2185.49%	0.005
<i>Veillonella</i>	0.11%	0.05%	2.58%	-57.41%	0.69	5547.37%	0.39	2305.27%	0.49
U. m. of Gammaproteobacteria class	0.00%	0.01%	0.11%	144.16%	0.38	928.75%	0.02	2411.74%	0.002
<i>Helicobacter</i>	0.03%	0.04%	0.97%	62.89%	0.9	2149.13%	0.02	3563.49%	0.03
U. m. of Enterobacteriaceae family	0.05%	0.06%	2.41%	20.68%	0.84	3770.70%	0.02	4571.27%	0.009
U. m. of Enterococcaceae family	0.01%	0.03%	0.46%	192.58%	0.11	1623.84%	0.008	4943.66%	0.02
<i>Enterococcus</i>	0.27%	1.95%	16.86%	629.45%	0.03	765.98%	0.0008	6216.91%	0.0005
<i>Staphylococcus</i>	0.01%	0.02%	0.64%	184.36%	0.39	2867.41%	0.09	8338.06%	0.2
<i>Klebsiella</i>	0.12%	0.19%	14.31%	54.25%	0.48	7429.84%	0.008	11514.90%	0.002
<i>Corynebacterium</i>	0.00%	0.00%	0.25%	-12.20%	0.2	21185.17%	0.3	18588.67%	0.72
<i>Hafnia</i>	0.00%	0.00%	0.19%	-83.81%	0.19	129552.00%	0.31	20894.04%	0.28
<i>Citrobacter</i>	0.01%	0.01%	4.39%	169.45%	0.67	30311.42%	0.35	81844.29%	0.25
<i>Proteus</i>	0.00%	0.00%	0.99%	114.82%	0.27	96585.45%	0.39	207601.83%	0.33

\* Age- and sex-adjusted

° Taxa in bold show decrease lower than 50% in CDI respect to AB- subjects with p-value <0.005

\* Taxa underlined show decrease lower than 50% or increase higher than 100% in AB+ and CDI respect to AB- subjects

U.m. = Unclassified members.

1

2

1

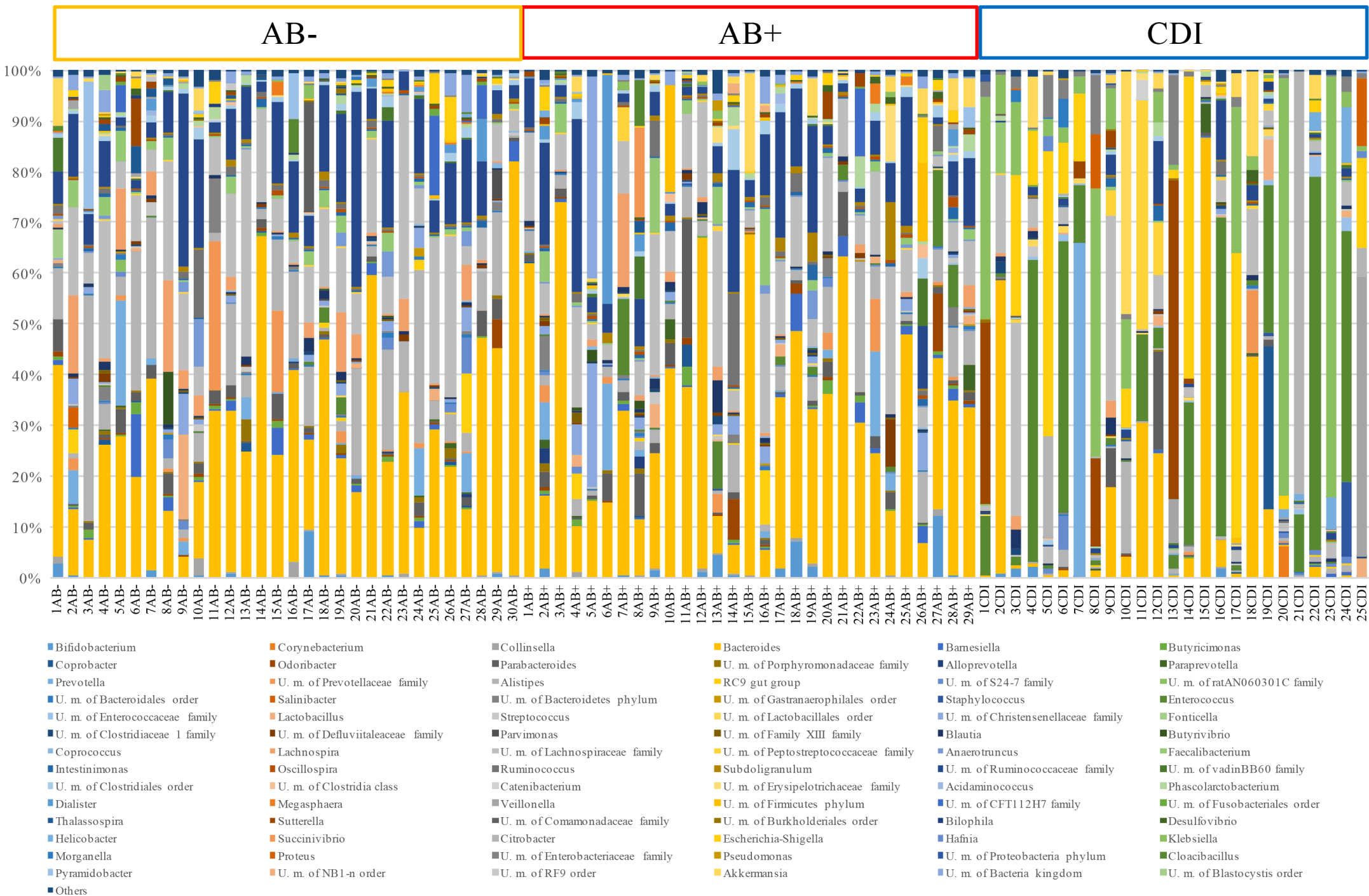
**Table S3.** Shotgun metagenomics datasets filtering table.

<b>Sample</b>	<b>Number of reads</b>	<b>Final read number</b>
7AB+	454948	167574
17AB+	817736	660670
18AB+	858782	508302
27AB+	533960	345756
28AB+	470413	372703
4CDI	594428	496810
6CDI	217508	132353
9CDI	608073	527978
14CDI	685437	191471
19CDI	500078	391433
4AB-	142807	121962
12AB-	225933	175843
18AB-	914260	209705
19AB-	215631	164462
22AB-	667180	571514

2

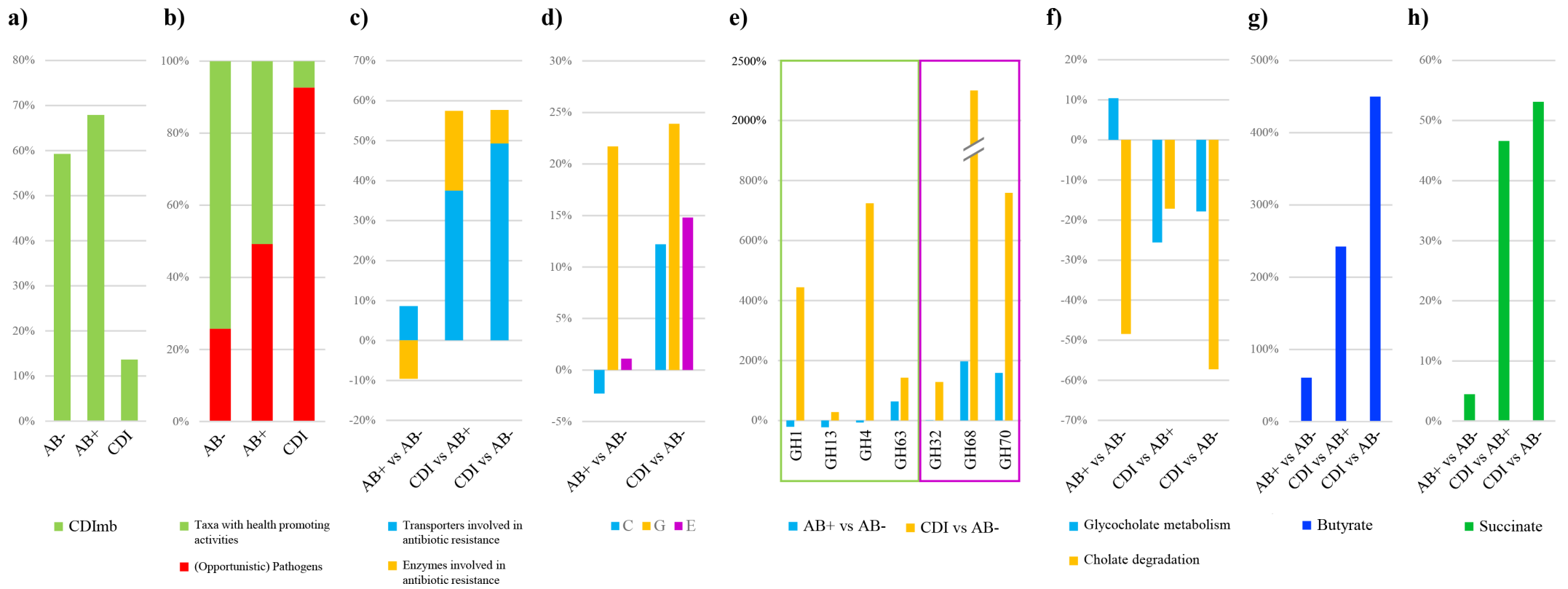
3





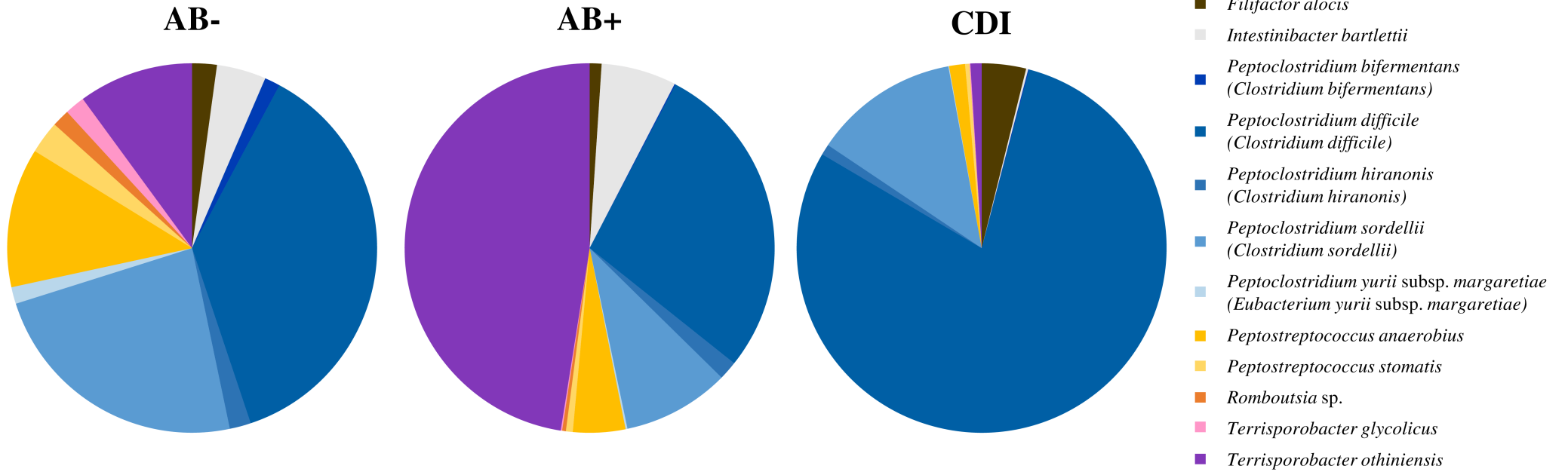
\* U. m. of = Unclassified member of

Figure S1

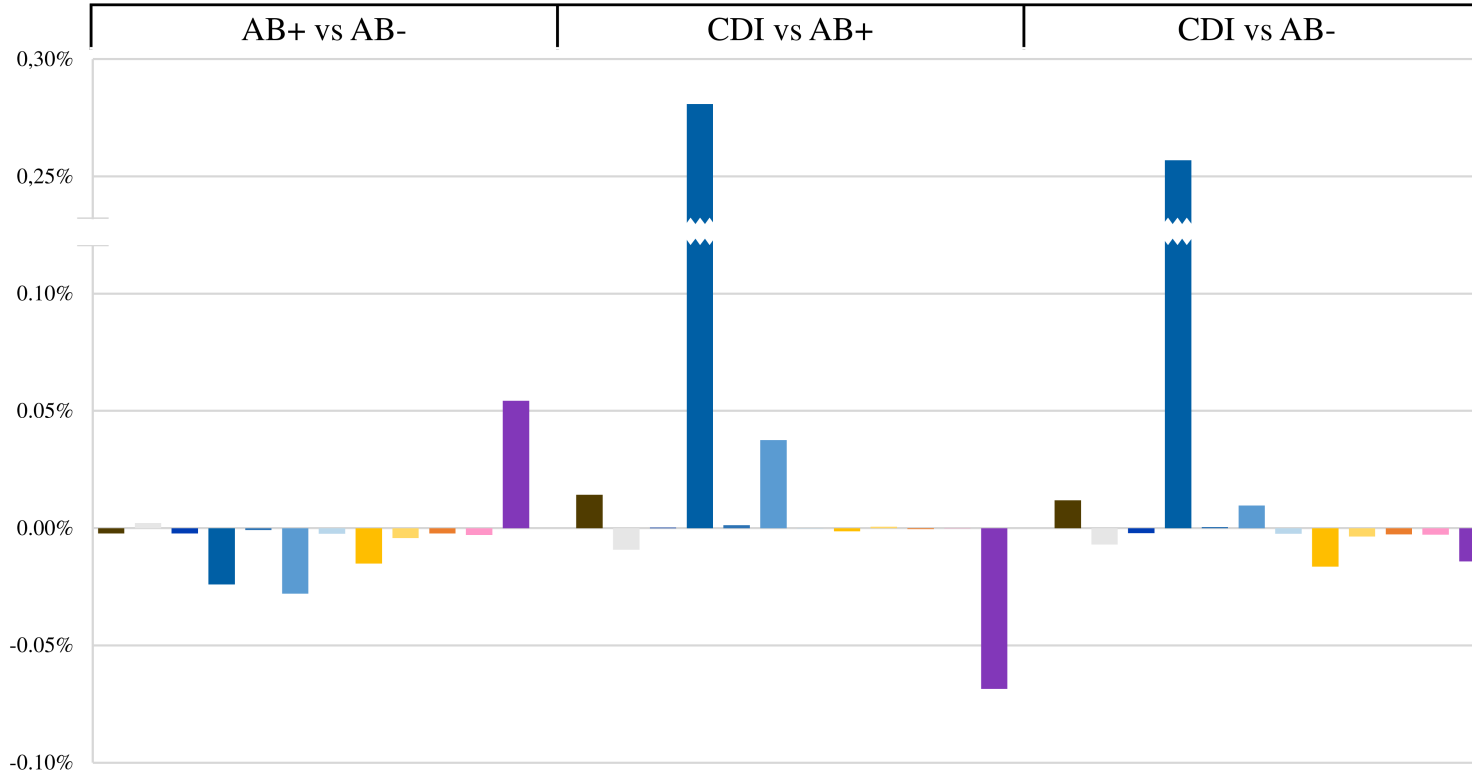


**Figure S2**

a)



b)



c)

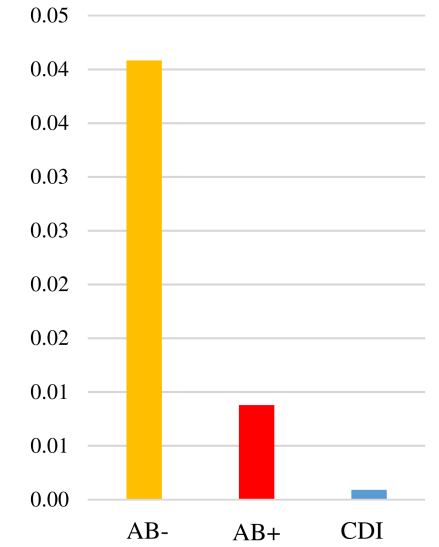


Figure S3