

Mycoplasma pneumoniae and *Streptococcus pneumoniae* caused different microbial structure and correlation network in lung microbiota

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Abstract: Pneumonia is one of the most serious diseases for children, with which lung microbiota are proved to be associated. We performed 16S rDNA analysis on broncho-alveolar lavage fluid (BALF) for 32 children with tracheomalacia (C group), pneumonia infected with *Streptococcus pneumoniae* (*S. pneumoniae*) (D1 group) or *Mycoplasma pneumoniae* (*M. pneumoniae*) (D2 group). Children with tracheomalacia held lower microbial diversity and accumulated *Lactococcus* (mean \pm SD, 45.21% \pm 5.07%, P value <0.05), *Porphyromonas* (0.12% \pm 0.31%, P value <0.05). D1 and D2 group were enriched by *Streptococcus* (7.57% \pm 11.61%, P value <0.01 when compared with D2 group) and *Mycoplasma* (0.67% \pm 1.25%, P value <0.01) respectively. Bacterial correlation in C group was mainly intermediated by *Pseudomonas* and *Arthrobacter*. Whilst, D1 group harbored simplest microbial correlation in three groups, and D2 group held the most complicated network, involving enriched *Staphylococcus* (0.26% \pm 0.71%), *Massilia* (0.81% \pm 2.42%). This will be of significance for understanding pneumonia incidence and progression more comprehensively, and discerning between bacterial infection and carriage.

Keywords: Pneumonia; *Streptococcus pneumoniae* (*S. pneumoniae*); *Mycoplasma pneumoniae* (*M. pneumoniae*); microbiota

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Background

Pneumonia is one of the most common illness in children, with high morbidity and mortality (1). Previous reports implicated that bacterial pathogens were the main contributors to pneumonia incidence and progression (2), and polymicrobial interaction was demonstrated, including *Mycoplasma pneumoniae* (*M. pneumoniae*), *Chlamydomphila pneumoniae* (*C. pneumoniae*), *Legionella pneumophila* (*L. pneumophila*), *Candida albicans* (*C. albicans*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) (2).

Besides above-mentioned pathogens, lung was home to

many kinds of bacteria (3,4). However, most of microbes in lung microbiota could not be cultured through conventional clinical detection. This will make it difficult to get the whole-picture of microbial interaction in the lung, and decrease the cure rate when uncultured or unknown lung pathogens existed. Culture-independent sequencing technology had been proved feasible in microbiota research on ventilator-associated, HIV-infected and idiopathic interstitial pneumonia (5-8). However, there was little report on microbial correlation network in microbiota at BALF, making it difficult to discern between bacterial

Table 1 Sample information for each subject

Grouping	C	D1	D2
Sample number	12	11	11
Median age (months)	6	10	60
Symptoms			
Fever	0	7	9
Cough	0	10	11
Persistent wheezing	6	3	2
Iconography			
Hyperinflation, unilateral emphysema	12	0	0
Lung consolidation, atelectasis, infiltration	0	11	11
Pathogen examination			
<i>S. pneumoniae</i> culture	0	11	0
<i>M. pneumoniae</i> PCR detection	0	0	11
Virus detection (influenza virus A/B, parainfluenza virus 1/2/3, adenovirus, respiratory syncytial virus)	0	0	0

PCR, polymerase chain reaction.

carriage and infection.

Considering above-mentioned background, we conducted BALF sampling on 34 children with tracheomalacia or pneumonia and performed 16S rDNA analysis for 32 samples. Several issues that we intended to resolve were: (I) discrepancy of bacterial structure in children with tracheomalacia and pneumonia infected with different pathogens? (II) If C, D1 and D2 groups harbored discrepant bacterial correlation and how? This will improve understanding involvement of lung microbiota in pneumonia etiology, and distinct microbial correlation under pneumonia infected with *S. pneumoniae* and *M. pneumoniae*. Based on these findings, further analysis of mechanisms behind these correlations will promote more precise diagnosis and treatment of pneumonia.

Methods

Sampling, DNA preparation and sequencing

Parents of all children patients wrote the consent and this study was also approved by the Ethical Committee of Shenzhen Children's Hospital under approval number

20150409436. Patients who fulfilled the following criteria were selected in this study: diagnosed with tracheomalacia, *M. pneumoniae* or *S. pneumoniae* pneumonia with characteristic chest radiographic abnormalities, patient symptoms, and clinical laboratory data (detailed in *Table 1*). BALF was collected by fiberoptic bronchoscopy and stored at -80°C .

Microbial DNA was extracted using the E.Z.N.A.[®] Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to manufacturer's protocols. DNA detection, amplification of 16S rDNA V3-V4 region and sequencing was performed as prior report (9). Raw reads were deposited in NCBI Sequence Read Archive (SRA) database (Accession Number: SRP067201).

Data processing and bioinformatics analysis

Raw data was filtered using QIIME (version 1.17) (10): (I) the reads were removed if successive 50 bases held <20 quality score; (II) filtered reads with >2 mismatched bases in primer were also removed; (III) paired-end reads with >10 bases overlap were selected to assemble tags. Removing redundancy of assembled tags, species annotation, structural diversity and comparative analysis among three groups was conducted following previous report (11). Bacterial taxa with relative abundance $>0.1\%$ were selected to evaluate microbial correlation. The correlation graph was produced by Cytoscape (v3.3.0) under >0.9 Pearson index (12).

Results

Sample information and grouping

Totally 12 subjects had tracheomalacia (C group), 11 children were diagnosed to be infected by *S. pneumoniae* (D1 group) and other 11 with severe pneumonia were infected with *M. pneumoniae* (D2 group). Two samples were removed from subsequent analysis due to abnormal microbial structure, dramatically enriched by 44% *Mycoplasma* and 92% *Haemophilus* respectively. Other 32 samples were summarized in *Table S1*.

Microbial structure is discrepant among three groups

Totally 14,568 clean tags were produced for each sample on average, ranging from 9,383 to 18,946. At the phylum level, Firmicutes and Proteobacteria accounted for more than 90% in all samples, except the sample 1,037 which held 27.17% unclassified phylum. *Lactococcus*, *Bacillus*, *Solibacillus*,



Figure 1 Bacterial structure in C, D1 and D2 group, at genus level. SVG (version 1.1) was used to produce the paragraph, based on relative abundance of each genus. The circle square of each genus represents total relative abundance in three groups, and the line linking C, D1 and D2 group means relative abundance for each group (proportional to line thickness). SVG, Scalable Vector Graphics.

Pseudomonas, *Streptococcus*, *Arthrobacter*, *Psychrobacter* and *Exiguobacterium* were the top eight genus in three groups (Tables S2-S4), representing more than 90% relative abundance. It was difficult to discriminate the three groups based on principal components analysis (PCA), but several genus were differentially distributed apparently (Figure 1). By comparison to C group, children from D1 group harbored more abundant *Streptococcus* ($7.51\% \pm 11.61\%$) and *Propionibacterium* ($0.24\% \pm 0.62\%$), less abundant *Lactococcus* ($40.50\% \pm 5.69\%$), *Neisseria* ($0.05\% \pm 0.14\%$) and *Moraxella* (almost 0) (P value was summarized in Tables S2-S4). *Propionibacterium* ($0.19\% \pm 0.43\%$), *Mycoplasma* ($0.67\% \pm 1.25\%$), *Massilia* ($0.81\% \pm 2.42\%$) and *Staphylococcus* ($0.26\% \pm 0.71\%$) were enriched in D2 group, when compared to C group (P value was summarized in Tables S2-S4). C group harbored lower microbial diversity by comparison to D1 and D2 group (Figure 2).

Different microbial correlation among C, D1 and D2 group

For children in C group, *Pseudomonas* and *Arthrobacter* were the core of correlation, with positive association to other normal colonizers, involving *Bacillus*, *Solibacillus*, *Psychrobacter*, *Flavobacterium*, *Exiguobacterium* and *Lactobacillus*, most of which were found to be the colonizers in the lung (4) [Figure 3 (C group)]. When children were infected with *S. pneumoniae*, the correlation network in C group was destroyed, and *Brochothrix*, *Janthinobacterium* were involved in different correlation independently [Figure 3 (D1 group)]. The most complicated correlation was found in D2 group, and three strong correlation links were constructed [Figure 3 (D2 group)]. Enriched *Staphylococcus*, *Propionibacterium* and *Massilia* intended to build intensive association with *Raoultella*, *Limnobabitans* and *Acinetobacter* [Figure 3 (D2 group)]. *Granulicatella* mediated

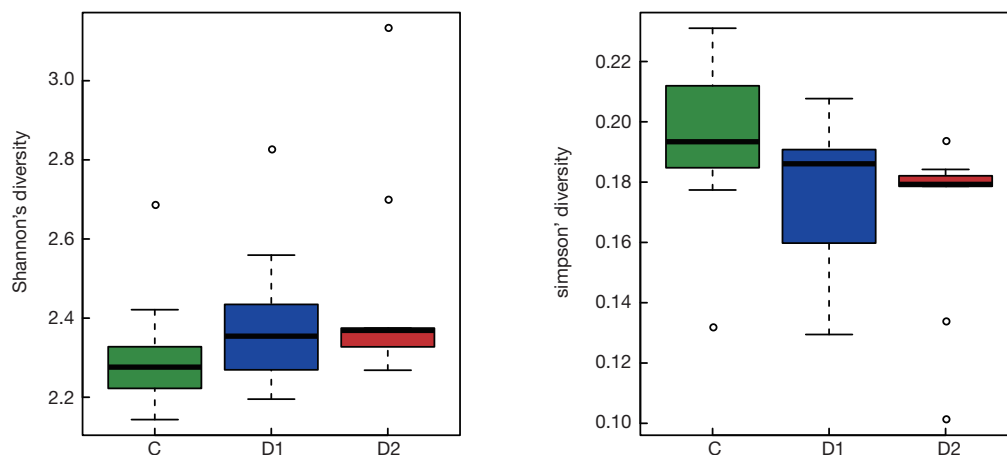


Figure 2 Alpha-diversity for microbial in C, D1 and D2 group. Shannon's diversity is proportional to microbial diversity, and Simpson's diversity is negatively correlated with diversity. Based on the two indexes, C group hold lower microbial diversity.

second network, involving *Streptococcus*, *Porphyromonas*, *Bacteroides* and *Prevotella* [Figure 3 (D2 group)]. The core of the third network was the most abundant *Lactococcus*, correlating positively with *Bacillus*, *Pseudomonas*, *Solibacillus* and *Psychrobacter* [Figure 3 (D2 group)].

Discussion

Increasing reports documented that respiratory tract is home to various microbes, which were associated with respiratory health (4). Sputum microbiota was demonstrated to be not concordant to microbes in the lung (13,14), and lung tissue could be collected only when lung transplantation will be conducted (3). This drove BALF sampling to be preferable strategy, in evaluating lung microbiota and corresponding involvement in lung diseases. We collected 32 samples with three symptoms: tracheomalacia, pneumonia infected with *S. pneumoniae*, pneumonia infected with *M. pneumoniae*. Previously identified microbial colonizers of lung, including *Lactococcus*, *Pseudomonas*, *Prevotella*, *Fusobacteria*, *Veillonella*, *Haemophilus* and *Neisseria* (15), were also found in this study, accounting for more than 90% of lung microbiota. Other pneumonia patients enriched bacterial species (5-8), including *Staphylococcus* and *Streptococcus*, were also increased in D1 and D2 group. Meanwhile, children with tracheomalacia harbored lower microbial diversity. This could be attributable to inhibitory effect of *S. pneumoniae* and *M. pneumoniae* on growth of naturally colonized microbes as well as more pathogen intrusion, and inter-individual difference which was demonstrated in intensive care unit (ICU) pneumonia patients (7).

The most interesting finding was that microbial correlation was totally different among three groups. In C group, *Pseudomonas* and *Arthrobacter* were in the core of interaction network and built positive relationship with other bacterial residents. *Pseudomonas* carriage was documented to be normal in prior reports (4), and this correlation also implicated homeostasis of microbiota at BALF, even if children from C group had tracheomalacia. When children were diagnosed pneumonia infected by *S. pneumoniae*, linkage of *Pseudomonas* and *Arthrobacter* to other bacterial colonizers were implicated to be broken up. This could be due to attachment of respiratory epithelial cells and evading host defenses by *S. pneumoniae* (16) through abundant various virulence factors like polysaccharide capsule and hydrogen peroxide, suggesting inhibitory effect of *S. pneumoniae* on other bacterial counterparts like *S. aureus* (17,18). Enrichment of *S. pneumoniae* in D1 group was also supported by clinical diagnosis based on traditional culture, and detection of *M. pneumoniae* in D2 group by single real time PCR, implicating accuracy of analysis results.

On the contrary, children infected by *M. pneumoniae* harbored more complicated bacterial correlation network. Besides *Lactococcus* and *Granulicatella* mediated network, D2 group enriched *Staphylococcus*, *Propionibacterium* and *Massilia* indicated to construct a complex positive interaction with *Acinetobacter*, *Raoultella* and *Limnobacter*. However, *Mycoplasma* seemed to be outside of these relationships, possibly attributable to lack of antagonistic relationship between *M. pneumoniae* with other airway pathogens, and low immunity of pneumonia children in D2 group caused by

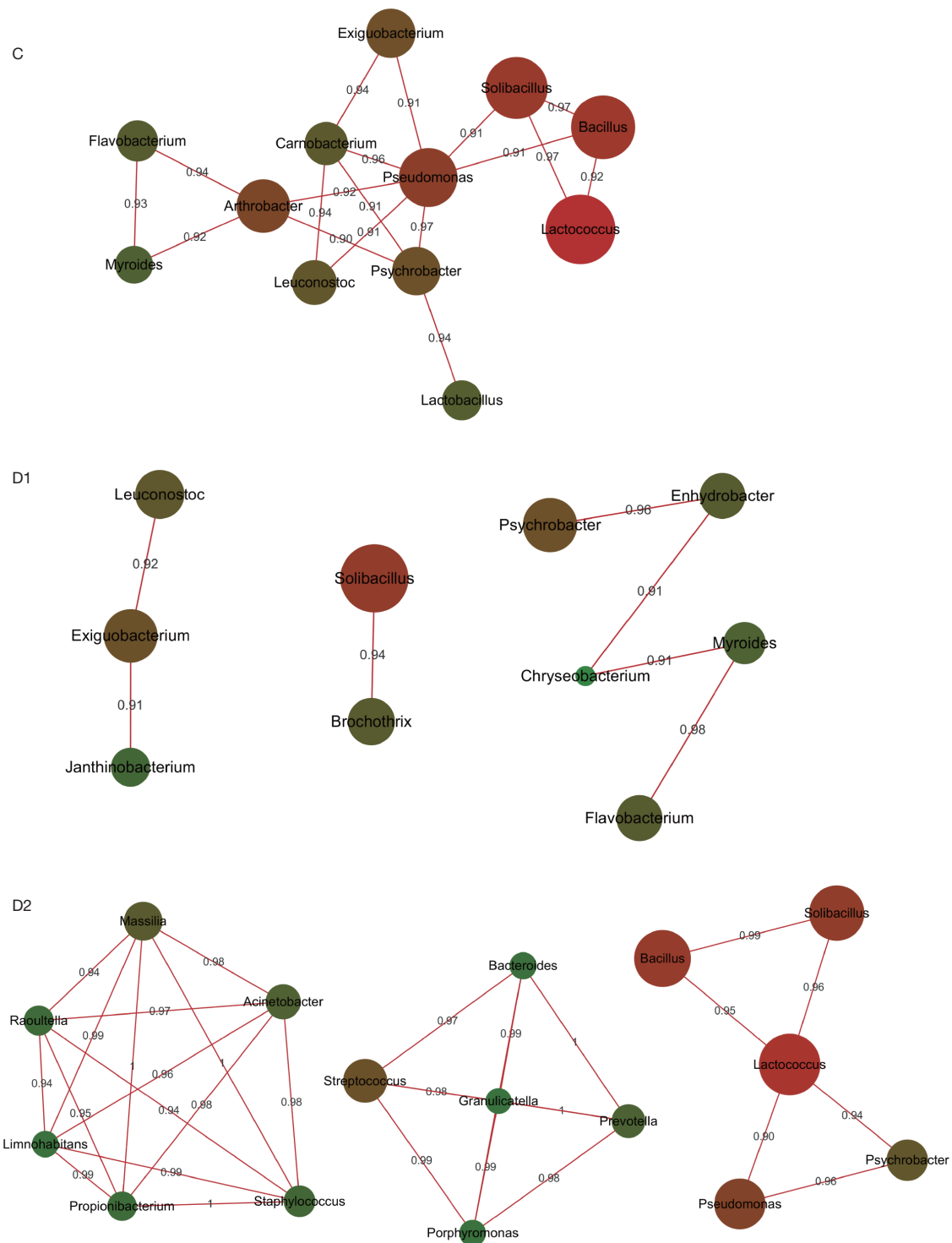


Figure 3 Bacterial correlation in C, D1 and D2 group. Microbial species with >0.1% relative abundance were selected to analyze correlation network, with Pearson index >0.9. The correlation network was mainly mediated by *Pseudomonas* and *Arthrobacter*, involving most of normal colonizers. When children got pneumonia, interaction network in C group were broken up: simpler in D1 group and more complicated in D2 group with another tight correlation circle constituted by enriched *Staphylococcus*, *Massilia* and *Propionibacterium*. The circle square is proportional to relative abundance, red line means positive correlation.

M. pneumoniae induced severe symptoms (19). And *S. aureus* was proved to be the airway pathogen, interacting with *S. pneumoniae*, *H. influenzae* and *P. aeruginosa* negatively (2), which could promote colonization of *S. aureus* at BALF of children infected with *M. pneumoniae*. However, *Massilia* will need more study to unravel its potential involvement in pneumonia inducement and progression, because there were just several reports about *Massilia* contribution to eye and skin illness (20).

Previous reports indicated that *S. pneumoniae*, *S. aureus*, *P. aeruginosa* and *H. influenzae* were also the main pathogenic agents for and accumulated in children with respiratory diseases (2-4). In this study, *Pseudomonas* and *Haemophilus* showed no discrepancy among three groups. We could not know if it was because children in C group were not totally healthy, or most of previous reports conducted different sampling. Other limitations of this study were insignificant discrepancy of some genera, including *Porphyromonas* and *Psychrobacter*, and ambiguous contribution of antibiotic, gender, age to difference among three groups. However, bacterial correlation in these groups will provide new insights into etiology of tracheomalacia and pneumonia infected with different bacterial pathogens.

To promote clinical application of these findings, several issues will be considered in our next step. Firstly, larger cohort study will be performed, and animal model will be collected to confirm and elucidate interaction network and mechanisms behind it. Then we will test if this could improve discerning between bacterial infection and carriage, with the aid of these correlation networks. Collectively, these findings will be applied in making more precise diagnosis and treatment on CAP, to lower the mortality and avoid overuse of antibiotic, including first-line macrolides (16).

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Supplementary

Table S1 Detailed information for each sample

Sample number	Gender	Age (years)	Number of days with symptom prior to hospitalization		Antibiotic use	Disease category
			Fever	Cough		
1544	Male	0.5	7	10	Penicillin; erythromycin	Tracheomalacia
1048	Male	0.3	0	0	Erythromycin	Tracheomalacia
1037	Male	0.4	0	>31	Penicillin	Tracheomalacia
1131	Male	1	0	4	Penicillin	Tracheomalacia
994	Male	0.7	1	3	Cephalosporins	Tracheomalacia
988	Female	0.8	5	2	Penicillin	Tracheomalacia
1086	Male	0.5	0	0	N	Tracheomalacia
1056	Male	0.5	0	20	N	Tracheomalacia
1577	Male	1	0	10	Cephalosporins	Tracheomalacia
1446	Female	0.4	0	10	Penicillin; macrolide	Tracheomalacia
1272	Male	0.4	0	>31	Cephalosporins; erythromycin	Tracheomalacia
1227	Male	2.9	4	>31	Erythromycin	Tracheomalacia
1444	Male	5	9	9	Macrolide	<i>M. Pneumoniae</i> pneumonia
1433	Female	8	0	2	Macrolide	<i>M. Pneumoniae</i> pneumonia
1431	Male	8	12	8	Macrolide	<i>M. Pneumoniae</i> pneumonia
1422	Female	5	5	5	Macrolide	<i>M. Pneumoniae</i> pneumonia
1347	Female	3	0	20	Macrolide	<i>M. Pneumoniae</i> pneumonia
1327	Male	9	7	7	Erythromycin	<i>M. Pneumoniae</i> pneumonia
1292	Male	5	7	7	Macrolide	<i>M. Pneumoniae</i> pneumonia
1399	Female	3	0	2	Cephalosporins	<i>M. Pneumoniae</i> pneumonia
1740	Male	4.5	3	3	Erythromycin; cephalosporins	<i>M. Pneumoniae</i> pneumonia
1741	Male	7.6	5	7	Cephalosporins; macrolide	<i>M. Pneumoniae</i> pneumonia
1216	Male	7	7	7	Penicillin; erythromycin; macrolide; cephalosporins	<i>M. Pneumoniae</i> pneumonia
1244	Male	3	4	>31	Erythromycin	<i>S. Pneumoniae</i> pneumonia
1211	Male	0.9	4	>31	Erythromycin	<i>S. Pneumoniae</i> pneumonia
1579	Male	0.8	0	>31	Cephalosporins; macrolide	<i>S. Pneumoniae</i> pneumonia
1080	Male	1	5	5	Erythromycin	<i>S. Pneumoniae</i> pneumonia
1161	Male	0.8	2	2	Penicillin; cephalosporins	<i>S. Pneumoniae</i> pneumonia
1316	Male	0.8	3	7	N	<i>S. Pneumoniae</i> pneumonia
1270	Male	0.5	2	6	Erythromycin	<i>S. Pneumoniae</i> pneumonia
1302	Female	0.3	0	10	Cephalosporins; penicillin	<i>S. Pneumoniae</i> pneumonia
1228	Male	0.8	0	>31	Penicillin; macrolide	<i>S. Pneumoniae</i> pneumonia
1250	Male	0.4	2	0.5	N	<i>S. Pneumoniae</i> pneumonia
1282	Female	4.5	0	>31	Macrolide	<i>S. Pneumoniae</i> pneumonia

Table S2 C-D1 wilcox test

Genus	MEAN group C	SD group C	MEAN group D1	SD group D1	P value	FDR
Luteibacter	0.002	0.005	0.009	0.014	0.033	0.793
Lactococcus	45.21	5.068	40.501	5.69	0.037	0.793
Porphyromonas	0.123	0.308	0.063	0.029	0.037	0.793
Chryseobacterium	0.065	0.026	0.101	0.053	0.037	0.793
Propionibacterium	0.024	0.015	0.241	0.618	0.051	0.793
Desemzia	0.012	0.01	0.006	0.011	0.054	0.793
Arthrobacter	2.999	0.585	2.514	0.634	0.059	0.793
Anoxybacillus	0.012	0.009	0.018	0.009	0.079	0.793
Staphylococcus	0.06	0.175	0.071	0.129	0.101	0.793
Anaerococcus	0	0.002	0.004	0.008	0.105	0.793
Butyricoccus	0.001	0.004	0.014	0.029	0.125	0.793
Phenyllobacterium	0.006	0.007	0.002	0.003	0.133	0.793
Gemella	0.002	0.004	0.006	0.011	0.141	0.793
Tessaracoccus	0.002	0.006	0.011	0.025	0.147	0.793
Demequina	0	0	0.002	0.005	0.148	0.793
Fusobacterium	0	0	0.001	0.003	0.148	0.793
Wautersiella	0	0	0.004	0.011	0.148	0.793
Streptococcus	2.706	2.18	7.571	11.608	0.151	0.793
Acinetobacter	0.318	0.091	0.508	0.383	0.151	0.793
Blautia	0.002	0.004	0	0	0.186	0.854
Nitrosomonas	0.001	0.004	0	0	0.186	0.854
Psychrobacter	1.232	0.179	1.567	0.618	0.211	0.854
Vibrio	0.002	0.004	0	0.002	0.284	0.854
Sphingomonas	0.001	0.002	0.003	0.007	0.284	0.854
Crocinitomix	0.005	0.008	0.006	0.017	0.29	0.854
Eggerthella	0	0	0.001	0.005	0.338	0.854
Eubacterium	0	0	0.006	0.021	0.338	0.854
Fingoldia	0	0	0.002	0.008	0.338	0.854
Methyloversatilis	0	0	0.001	0.002	0.338	0.854
Mycoplasma	0	0	0.002	0.007	0.338	0.854
Parvimonas	0	0	0.001	0.005	0.338	0.854
Phyllobacterium	0	0	0.001	0.005	0.338	0.854
Ruminiclostridium	0	0	0.003	0.01	0.338	0.854
Schwartzia	0	0	0.005	0.018	0.338	0.854
Myroides	0.205	0.061	0.233	0.203	0.347	0.854
Janthinobacterium	0.139	0.069	0.169	0.075	0.379	0.854
Limnohabitans	0.041	0.019	0.056	0.033	0.379	0.854
Unclassified	0.001	0.004	0	0	0.384	0.854
Mycobacterium	0.001	0.003	0	0	0.384	0.854
Bradyrhizobium	0.001	0.003	0	0	0.384	0.854
Alloprevotella	0.001	0.002	0	0	0.384	0.854
Pirellula	0.001	0.002	0	0	0.384	0.854
Pseudonocardia	0	0.002	0	0	0.384	0.854
Haliaea	0	0.002	0	0	0.384	0.854
Peptostreptococcus	0	0.002	0	0	0.384	0.854
Pseudomonas	7.034	0.933	8.178	2.999	0.413	0.871
Altererythrobacter	0.001	0.002	0.002	0.004	0.418	0.871
Lysobacter	0.001	0.002	0.011	0.032	0.418	0.871
Oceanobacillus	0.011	0.017	0.007	0.016	0.434	0.874
Neisseria	0.763	2.629	0.049	0.136	0.447	0.874
Paenibacillus	0.015	0.014	0.019	0.013	0.46	0.874
Devosia	0	0.002	0.001	0.002	0.462	0.874
Bacillus	16.732	1.82	17.162	4.288	0.487	0.874
Carnobacterium	0.502	0.094	0.45	0.104	0.487	0.874
Corynebacterium	0.029	0.064	0.014	0.023	0.489	0.874
Haemophilus	0.045	0.121	0.008	0.014	0.492	0.874
Pseudoalteromonas	0.003	0.004	0.002	0.006	0.498	0.874
Massilia	0.002	0.004	0.002	0.004	0.58	0.999
Veillonella	0.081	0.185	0.032	0.078	0.59	0.999
Unclassified_Armatimonadetes	0.003	0.006	0.001	0.003	0.609	1
Megasphaera	0.007	0.016	0.003	0.011	0.636	1
Hydrogenophilus	0.002	0.003	0.007	0.017	0.661	1
Bacteroides	0.063	0.175	0.019	0.018	0.676	1
Uruburuella	0.081	0.03	0.084	0.034	0.695	1
Actinomyces	0.002	0.005	0.005	0.016	0.713	1
Sandaracinobacter	0.001	0.002	0.002	0.006	0.713	1
Dokdonella	0.005	0.013	0.003	0.007	0.733	1
Ignavibacterium	0.003	0.005	0.002	0.005	0.733	1
Nitrospira	0.009	0.023	0.006	0.011	0.745	1
Comamonas	0.02	0.019	0.031	0.038	0.758	1
Ferribacterium	0.009	0.016	0.008	0.017	0.78	1
Psychromonas	0.005	0.013	0.012	0.031	0.78	1
Exiguobacterium	1.423	0.211	1.477	0.467	0.786	1
Peptoniphilus	0.002	0.004	0.002	0.004	0.798	1
Brevundimonas	0.016	0.012	0.06	0.143	0.829	1
Dechloromonas	0.017	0.031	0.016	0.022	0.871	1
Paracoccus	0.012	0.016	0.013	0.016	0.877	1
Acetobacter	0.015	0.014	0.015	0.017	0.877	1
Aquabacterium	0.011	0.009	0.011	0.011	0.877	1
Epilithonimonas	0.021	0.016	0.025	0.029	0.878	1
Enhydrobacter	0.401	0.104	0.484	0.227	0.88	1
Flavobacterium	0.36	0.092	0.456	0.337	0.88	1
Petrobacter	0.003	0.008	0.003	0.006	0.905	1
Facklamia	0.005	0.005	0.01	0.022	0.922	1
Ochrobactrum	0.006	0.005	0.019	0.047	0.925	1
Leuconostoc	0.67	0.14	0.714	0.279	0.928	1
Brochothrix	0.567	0.091	0.561	0.126	0.928	1
Lactobacillus	0.298	0.06	0.289	0.071	0.928	1
Parabacteroides	0.001	0.002	0.001	0.002	0.95	1
Steroidobacter	0.001	0.002	0.001	0.003	0.95	1
Aeromonas	0.004	0.013	0.001	0.003	0.963	1
Unclassified_Acidobacteria	0.002	0.004	0.003	0.008	0.963	1
Clostridium	0.001	0.003	0.003	0.008	0.963	1
Solibacillus	12.538	1.18	12.744	2.202	0.976	1
Raoultella	0.182	0.072	0.247	0.26	0.976	1
Moraxella	0.339	1.176	0.002	0.006	1	1
Prevotella	0.141	0.287	0.092	0.21	1	1
Granulicatella	0.043	0.022	0.045	0.029	1	1
Sphingobacterium	0.015	0.009	0.017	0.013	1	1
Thauera	0.015	0.019	0.012	0.012	1	1

Table S3 C-D2 wilcox test

Genus	MEAN group C	SD group C	MEAN group D2	SD group D2	P value	FDR
Lactococcus	45.21	5.068	40.825	4.632	0.006	0.148
Bacillus	16.732	1.82	18.566	1.781	0.058	0.499
Solibacillus	12.538	1.18	14.276	1.313	0.006	0.148
Pseudomonas	7.034	0.933	7.463	1.052	0.464	0.799
Arthrobacter	2.999	0.585	2.693	0.332	0.148	0.555
Streptococcus	2.706	2.18	2.167	1.939	0.069	0.499
Exiguobacterium	1.423	0.211	1.56	0.206	0.148	0.555
Psychrobacter	1.232	0.179	1.451	0.185	0.018	0.322
Neisseria	0.763	2.629	0.065	0.118	0.071	0.499
Leuconostoc	0.67	0.14	0.718	0.126	0.422	0.791
Brochothrix	0.567	0.091	0.545	0.07	0.702	0.932
Carnobacterium	0.502	0.094	0.463	0.064	0.382	0.787
Enhydrobacter	0.401	0.104	0.501	0.106	0.111	0.521
Flavobacterium	0.36	0.092	0.365	0.103	0.972	1
Moraxella	0.339	1.176	0.014	0.04	0.484	0.819
Acinetobacter	0.318	0.091	0.517	0.361	0.034	0.432
Lactobacillus	0.298	0.06	0.292	0.04	0.808	0.932
Myroides	0.205	0.061	0.227	0.063	0.808	0.932
Raoultella	0.182	0.072	0.212	0.159	0.808	0.932
Prevotella	0.141	0.287	0.372	0.899	0.565	0.891
Janthinobacterium	0.139	0.069	0.167	0.04	0.095	0.521
Porphyromonas	0.123	0.308	0.118	0.193	0.095	0.521
Veillonella	0.081	0.185	0.078	0.141	0.684	0.932
Uruburuella	0.081	0.03	0.081	0.037	0.651	0.932
Chryseobacterium	0.065	0.026	0.099	0.072	0.219	0.692
Bacteroides	0.063	0.175	0.126	0.284	0.529	0.868
Staphylococcus	0.06	0.175	0.26	0.706	0.08	0.51
Haemophilus	0.045	0.121	0.043	0.08	0.115	0.521
Granulicatella	0.043	0.022	0.108	0.219	0.651	0.932
Limnohabitans	0.041	0.019	0.116	0.229	0.651	0.932
Corynebacterium	0.029	0.064	0.053	0.146	0.171	0.619
Propionibacterium	0.024	0.015	0.187	0.429	0.006	0.148
Epilithonimonas	0.021	0.016	0.026	0.016	0.455	0.796
Comamonas	0.02	0.019	0.017	0.016	0.748	0.932
Dechloromonas	0.017	0.031	0.013	0.025	0.723	0.932
Brevundimonas	0.016	0.012	0.066	0.124	0.07	0.499
Sphingobacterium	0.015	0.009	0.017	0.026	0.354	0.776
Paenibacillus	0.015	0.014	0.012	0.008	0.754	0.932
Acetobacter	0.015	0.014	0.007	0.009	0.138	0.555
Thauera	0.015	0.019	0.015	0.038	0.244	0.692
Anoxybacillus	0.012	0.009	0.012	0.011	0.858	0.932
Desemzia	0.012	0.01	0.019	0.015	0.371	0.78
Paracoccus	0.012	0.016	0.023	0.055	0.328	0.766
Oceanobacillus	0.011	0.017	0.036	0.077	0.355	0.776
Aquabacterium	0.011	0.009	0.096	0.273	0.694	0.932
Nitrospira	0.009	0.023	0.002	0.004	0.446	0.794
Ferribacterium	0.009	0.016	0.008	0.017	0.937	0.982
Megasphaera	0.007	0.016	0.001	0.002	0.683	0.932
Phenylobacterium	0.006	0.007	0.005	0.008	0.908	0.973
Ochrobactrum	0.006	0.005	0.012	0.025	0.796	0.932
Dokdonella	0.005	0.013	0	0	0.129	0.543
Crocinitomix	0.005	0.008	0.001	0.002	0.119	0.521
Psychromonas	0.005	0.013	0.003	0.008	0.5	0.834
Facklamia	0.005	0.005	0.004	0.005	0.94	0.982
Aeromonas	0.004	0.013	0.011	0.019	0.083	0.51
Petrobacter	0.003	0.008	0.004	0.005	0.397	0.791
Unclassified_Armatimonadetes	0.003	0.006	0.005	0.01	1	1
Ignavibacterium	0.003	0.005	0.007	0.015	0.689	0.932
Pseudoalteromonas	0.003	0.004	0.009	0.023	1	1
Luteibacter	0.002	0.005	0.004	0.011	0.796	0.932
Actinomyces	0.002	0.005	0.005	0.01	0.419	0.791
Vibrio	0.002	0.004	0.063	0.188	0.569	0.891
Peptoniphilus	0.002	0.004	0.003	0.008	0.569	0.891
Blautia	0.002	0.004	0	0	0.236	0.692
Tessaracoccus	0.002	0.006	0.002	0.006	0.944	0.982
Unclassified_Acidobacteria	0.002	0.004	0.034	0.075	0.038	0.432
Hydrogenophilus	0.002	0.003	0.002	0.004	1	1
Gemella	0.002	0.004	0.014	0.036	0.366	0.78
Massilia	0.002	0.004	0.808	2.423	0.861	0.932
Nitrosomonas	0.001	0.004	0.012	0.035	0.717	0.932
Clostridium	0.001	0.003	0.003	0.006	0.796	0.932
Butyricoccus	0.001	0.004	0	0	0.441	0.794
Unclassified	0.001	0.004	0	0	0.441	0.794
Altererythrobacter	0.001	0.002	0.006	0.019	0.861	0.932
Sandaracinobacter	0.001	0.002	0.028	0.083	0.717	0.932
Mycobacterium	0.001	0.003	0.003	0.008	0.414	0.791
Lysobacter	0.001	0.002	0.01	0.017	0.047	0.445
Bradyrhizobium	0.001	0.003	0.011	0.031	0.414	0.791
Sphingomonas	0.001	0.002	0.01	0.029	0.834	0.932
Alloprevotella	0.001	0.002	0.055	0.165	0.834	0.932
Parabacteroides	0.001	0.002	0.023	0.036	0.014	0.3
Steroidobacter	0.001	0.002	0.022	0.066	0.834	0.932
Pirellula	0.001	0.002	0.018	0.054	0.834	0.932
Anaerococcus	0	0.002	0.04	0.116	0.35	0.776
Pseudonocardia	0	0.002	0.001	0.004	0.834	0.932
Devosia	0	0.002	0.011	0.033	0.834	0.932
Peptostreptococcus	0	0.002	0.013	0.04	0.834	0.932
Halieta	0	0.002	0.001	0.004	0.834	0.932
Mycoplasma	0	0	0.673	1.25	0	0.046
Parvimonas	0	0	0.02	0.047	0.041	0.432
Fusobacterium	0	0	0.009	0.017	0.041	0.432
Ensifer	0	0	0.008	0.023	0.29	0.692
Gaiella	0	0	0.008	0.023	0.29	0.692
Sneathia	0	0	0.006	0.019	0.29	0.692
Rothia	0	0	0.005	0.015	0.29	0.692
Methyloversatilis	0	0	0.005	0.014	0.29	0.692
Chelatococcus	0	0	0.003	0.01	0.29	0.692
Treponema	0	0	0.003	0.01	0.29	0.692
Blastopirellula	0	0	0.003	0.008	0.29	0.692
Nocardioides	0	0	0.003	0.008	0.29	0.692
Thiobacillus	0	0	0.003	0.008	0.29	0.692
Finegoldia	0	0	0.003	0.005	0.109	0.521
Selenomonas	0	0	0.003	0.008	0.29	0.692
Wautersiella	0	0	0.002	0.004	0.109	0.521
Brevibacterium	0	0	0.001	0.004	0.29	0.692

Table S4 D1-D2 wilcox test

Genus	MEAN group D1	SD group D1	MEAN group D2	SD group D2	P value	FDR
Lactococcus	40.501	5.69	40.825	4.632	0.71	0.993
Bacillus	17.162	4.288	18.566	1.781	0.261	0.817
Solibacillus	12.744	2.202	14.276	1.313	0.112	0.817
Pseudomonas	8.178	2.999	7.463	1.052	0.941	1
Arthrobacter	2.514	0.634	2.693	0.332	0.824	1
Streptococcus	7.571	11.608	2.167	1.939	0.007	0.406
Exiguobacterium	1.477	0.467	1.56	0.206	0.882	1
Psychrobacter	1.567	0.618	1.451	0.185	1	1
Massilia	0.002	0.004	0.808	2.423	0.514	0.863
Leuconostoc	0.714	0.279	0.718	0.126	0.37	0.841
Mycoplasma	0.002	0.007	0.673	1.25	0.002	0.22
Brochothrix	0.561	0.126	0.545	0.07	0.71	0.993
Acinetobacter	0.508	0.383	0.517	0.361	0.882	1
Enhydrobacter	0.484	0.227	0.501	0.106	0.37	0.841
Carnobacterium	0.45	0.104	0.463	0.064	0.882	1
Prevotella	0.092	0.21	0.372	0.899	0.626	0.922
Flavobacterium	0.456	0.337	0.365	0.103	0.882	1
Lactobacillus	0.289	0.071	0.292	0.04	1	1
Staphylococcus	0.071	0.129	0.26	0.706	0.909	1
Myroides	0.233	0.203	0.227	0.063	0.201	0.817
Raoultella	0.247	0.26	0.212	0.159	0.941	1
Propionibacterium	0.241	0.618	0.187	0.429	0.882	1
Janthinobacterium	0.169	0.075	0.167	0.04	1	1
Bacteroides	0.019	0.018	0.126	0.284	0.877	1
Porphyromonas	0.063	0.029	0.118	0.193	1	1
Limnochobacter	0.056	0.033	0.116	0.229	0.656	0.953
Granulicatella	0.045	0.029	0.108	0.219	0.941	1
Chryseobacterium	0.101	0.053	0.099	0.072	0.412	0.841
Aquabacterium	0.011	0.011	0.096	0.273	0.564	0.898
Uruburuella	0.084	0.034	0.081	0.037	1	1
Veillonella	0.032	0.078	0.078	0.141	0.969	1
Brevundimonas	0.06	0.143	0.066	0.124	0.287	0.817
Neisseria	0.049	0.136	0.065	0.118	0.346	0.841
Vibrio	0	0.002	0.063	0.188	0.884	1
Alloprevotella	0	0	0.055	0.165	0.315	0.817
Corynebacterium	0.014	0.023	0.053	0.146	0.506	0.863
Haemophilus	0.008	0.014	0.043	0.08	0.203	0.817
Anaerococcus	0.004	0.008	0.04	0.116	0.743	1
Oceanobacillus	0.007	0.016	0.036	0.077	0.081	0.817
Unclassified_Acidobacteria	0.003	0.008	0.034	0.075	0.075	0.817
Sandaracinobacter	0.002	0.006	0.028	0.083	0.463	0.856
Epilithonimonas	0.025	0.029	0.026	0.016	0.569	0.898
Parabacteroides	0.001	0.002	0.023	0.036	0.025	0.817
Paracoccus	0.013	0.016	0.023	0.055	0.473	0.86
Steroidobacter	0.001	0.003	0.022	0.066	0.884	1
Parvimonas	0.001	0.005	0.02	0.047	0.192	0.817
Desemzia	0.006	0.011	0.019	0.015	0.056	0.817
Pirellula	0	0	0.018	0.054	0.315	0.817
Comamonas	0.031	0.038	0.017	0.016	0.594	0.912
Sphingobacterium	0.017	0.013	0.017	0.026	0.423	0.841
Thauera	0.012	0.012	0.015	0.038	0.203	0.817
Moraxella	0.002	0.006	0.014	0.04	0.463	0.856
Gemella	0.006	0.011	0.014	0.036	0.797	1
Peptostreptococcus	0	0	0.013	0.04	0.315	0.817
Dechloromonas	0.016	0.022	0.013	0.025	0.454	0.856
Ochrobactrum	0.019	0.047	0.012	0.025	0.75	1
Anoxybacillus	0.018	0.009	0.012	0.011	0.208	0.817
Paenibacillus	0.019	0.013	0.012	0.008	0.37	0.841
Nitrosomonas	0	0	0.012	0.035	0.126	0.817
Aeromonas	0.001	0.003	0.011	0.019	0.09	0.817
Devosia	0.001	0.002	0.011	0.033	0.807	1
Bradyrhizobium	0	0	0.011	0.031	0.126	0.817
Lysobacter	0.011	0.032	0.01	0.017	0.303	0.817
Sphingomonas	0.003	0.007	0.01	0.029	0.514	0.863
Fusobacterium	0.001	0.003	0.009	0.017	0.424	0.841
Pseudoalteromonas	0.002	0.006	0.009	0.023	0.549	0.893
Ferribacterium	0.008	0.017	0.008	0.017	0.83	1
Ensifer	0	0	0.008	0.023	0.315	0.817
Gaiella	0	0	0.008	0.023	0.315	0.817
Ignavibacterium	0.002	0.005	0.007	0.015	0.485	0.863
Acetobacter	0.015	0.017	0.007	0.009	0.224	0.817
Altererythrobacter	0.002	0.004	0.006	0.019	0.514	0.863
Sneathia	0	0	0.006	0.019	0.315	0.817
Phenylobacterium	0.002	0.003	0.005	0.008	0.327	0.828
Rothia	0	0	0.005	0.015	0.315	0.817
Actinomyces	0.005	0.016	0.005	0.01	0.277	0.817
Unclassified_Armatimonadetes	0.001	0.003	0.005	0.01	0.704	0.993
Methyloversatilis	0.001	0.002	0.005	0.014	0.884	1
Facklamia	0.01	0.022	0.004	0.005	0.934	1
Luteibacter	0.009	0.014	0.004	0.011	0.096	0.817
Petrobacter	0.003	0.006	0.004	0.005	0.593	0.912
Mycobacterium	0	0	0.003	0.008	0.126	0.817
Chelatococcus	0	0	0.003	0.01	0.315	0.817
Treponema	0	0	0.003	0.01	0.315	0.817
Clostridium	0.003	0.008	0.003	0.006	0.957	1
Psychromonas	0.012	0.031	0.003	0.008	0.447	0.856
Peptoniphilus	0.002	0.004	0.003	0.008	0.807	1
Blastopirellula	0	0	0.003	0.008	0.315	0.817
Nocardioides	0	0	0.003	0.008	0.315	0.817
Thiobacillus	0	0	0.003	0.008	0.315	0.817
Fingoldia	0.002	0.008	0.003	0.005	0.541	0.893
Selenomonas	0	0	0.003	0.008	0.315	0.817
Tessaracoccus	0.011	0.025	0.002	0.006	0.231	0.817
Hydrogenophilus	0.007	0.017	0.002	0.004	0.765	1
Wautersiella	0.004	0.011	0.002	0.004	0.957	1
Nitrospira	0.006	0.011	0.002	0.004	0.618	0.922
Haliaea	0	0	0.001	0.004	0.315	0.817
Pseudonocardia	0	0	0.001	0.004	0.315	0.817
Brevibacterium	0	0	0.001	0.004	0.315	0.817
Crocinitomix	0.006	0.017	0.001	0.002	0.625	0.922
Megasphaera	0.003	0.011	0.001	0.002	1	1
Butyricoccus	0.014	0.029	0	0	0.057	0.817
Eubacterium	0.006	0.021	0	0	0.421	0.841
Schwartzia	0.005	0.018	0	0	0.421	0.841
Dokdonella	0.003	0.007	0	0	0.215	0.817
Ruminiclostridium	0.003	0.01	0	0	0.421	0.841
Demequina	0.002	0.005	0	0	0.215	0.817
Eggerthella	0.001	0.005	0	0	0.421	0.841
Phyllobacterium	0.001	0.005	0	0	0.421	0.841