Published in final edited form as:

J Matern Fetal Neonatal Med. 2020 February ; 33(3): 359–367. doi:10.1080/14767058.2018.1490721.

Midtrimester Microbial DNA Variations in Maternal Serum of Women who Experience Spontaneous Preterm Birth

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Abstract

Objectives: To evaluate if midtrimester maternal serum contains microbial DNA and whether it differs between women with spontaneous preterm birth (SPTB) and those delivering at term.

Study Design: In this retrospective case-control study, we identified 20 healthy nulliparas with SPTB at 24–33 weeks of a non-anomalous singleton in 2014. Each case was matched by race/ethnicity to a control delivering at 39–40 weeks. Serum samples, collected at 15–20 weeks and stored at –80C, were thawed and DNA extracted. PCR with primers targeting the 16S rDNA V4 region were used to prepare an amplicon library, sequenced using Illumina MiSeq, and analyzed using QIIME. Taxonomy was assigned using RDP Classifier (threshold 0.8) against a modified Greengenes database. Differences in number of observed species, microbial alpha-diversity and beta-diversity, and taxa level analyses were undertaken.

Results: All 40 samples were included. Women with SPTB had more unique observed species ($p=0.046$) and higher mean alpha-diversity by Shannon index (but not Chao1 or Simpson) ($p=0.024$). Microbial composition was different between groups by Bray-Curtis clustering ($p=0.03$) but not by weighted ($p=0.13$) or unweighted Unifrac ($p=0.11$). Numerous taxa in the Firmicutes, Proteobacteria, and Actinobacteria phyla differed between groups ($p < 0.05$).

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The authors report no conflicts of interest.

This study was presented in part at the 37th Annual Meeting of the Society for Maternal-Fetal Medicine in Las Vegas, NV January 23–28, 2017

Conclusions: SPTB is associated with distinct microbial DNA changes detected in midtrimester maternal serum.

Keywords

Maternal serum; microbial DNA; midtrimester; spontaneous preterm birth

INTRODUCTION

Preterm birth (PTB; delivery before 37 weeks) is a leading cause of perinatal morbidity and mortality worldwide, with an annual cost of \$26.2 billion in the United States alone.^{1–3} While PTB may be indicated (due to maternal or fetal disease), 75% are spontaneous (SPTB), following preterm labor or premature rupture of the membranes (PROM).⁴ Infection is the only pathologic process for which SPTB causality has unequivocally been established, with most early (before 34 weeks) SPTBs due to overt or subclinical infection of the upper genital tract.^{4–7} The prevailing theory is that microorganisms ascend from the vagina into the upper genital tract causing a host inflammatory response that triggers ripening of the cervix, premature rupture of the membranes (PROM), and/or preterm labor.^{4–7} However, the identification and characterization of these microorganisms has been limited by traditional methods of bacterial culture.

New molecular sequencing technologies using 16S ribosomal DNA and whole genome sequencing (WGS) allow the microbial composition in a tissue (microbiome) to be more completely characterized. These techniques have not only been utilized to better describe the complex flora of the cervix/vagina in pregnancy but also to establish that tissues such as the placenta and amniotic fluid have a microbiome, and thus are not sterile.^{8–22} Furthermore, many of these studies have shown that there are alterations in these microbiomes in women who deliver preterm as compared to term – with many of these alterations detectable as early as 15 weeks of gestation.^{8–22}

Currently, measuring the cervical length by transvaginal ultrasound is the best available screening modality during pregnancy to detect women at risk for SPTB; however, its performance as a screening test is of limited utility, highlighting the need for better screening tools.²³ Maternal blood is routinely sampled during pregnancy for prenatal laboratory evaluation and represents an easily obtained tissue that could be used for screening purposes with no increase in discomfort over current standard prenatal laboratory testing.

Given findings of previous studies in other maternal microbial niches, we hypothesized that there may be microorganisms or residual microbial DNA in maternal serum and that variations in this microbial DNA may be associated with SPTB. Therefore, our objective was to evaluate if maternal serum collected in the midtrimester contains microbial DNA, and if so, to test for differences in the serum microbial DNA between women who have a SPTB and those who deliver at term.

MATERIALS AND METHODS

Sample Identification

In this IRB-approved (N140819001) retrospective case-control study, we utilized our obstetric database to identify 20 healthy primigravidas (in a blinded fashion) who delivered a non-anomalous singleton gestation via SPTB between 24–33 weeks' gestation in 2014 at the University of Alabama at Birmingham. Each patient was matched (1:1 matching) by race/ethnicity to a control delivering at 39–40 weeks during the same year. This sample size (20 cases and 20 controls) was pre-specified given the exploratory nature of our study. All patients were nulliparas with no prior pregnancies 14 weeks' gestation. Patients were excluded from this study for any of the following criteria: multiple gestation, chronic hypertension, collagen vascular disease, serum creatinine >1.1, pregestational diabetes, indicated PTB, fetal anomalies, or maternal age <19. Inclusion criteria as well as classification with a spontaneous preterm birth were confirmed with detailed chart review.

For these included patients, clinical remnant serum samples (~2mL) collected at 15–20 weeks as part of prenatal laboratory testing and stored at –80C were identified and retrieved from our departmental clinical laboratory biorepository. Of note, prior to this study, these samples had not been subjected to previous thawing.

Isolation of Microbial DNA and Creation of 16S V4 Amplicon Library.

Samples were thawed, and microbial genomic DNA was isolated from 200 microliters of maternal serum using the Fecal DNA isolation kit from Zymo Research (Irvine, CA) (catalog no. D6010) following the manufacturer's instructions. Once the sample DNA was prepared, PCR was used with unique bar coded primers to amplify the V4 region of the 16S ribosomal DNA (rDNA) to create an "amplicon library" from individual samples.^{24,25} Details of the V4 primers and our PCR protocol have been previously published.^{13,26,27}

DNA Sequencing and Bioinformatics

Samples were first quantitated using Pico Green (Molecular Probes, Inc., Eugene, OR), adjusted to a concentration of 4nM, and then used for sequencing.²⁸ The PCR amplicon covered approximately 255 bases of the V4 region of the 16S rDNA. All the samples were multiplexed using barcodes, and 251 base paired end reads were sequenced using the Illumina MiSeq platform (San Diego, CA).^{24,25} FASTQ conversion of the raw data files was performed following de-multiplexing. Quality assessment of the FASTQ files was performed using FASTQC, and then quality filtering was done using the FASTX toolkit.^{28,29} Since the overlap between fragments was approximately 245 bases, both paired reads were merged to generate a single high quality read using the module "fastq_mergepairs" of USEARCH (Drive 5). Read pairs with an overlap of less than 200 bases or with too many mismatches (>20) in the overlapping region were discarded. Chimeric sequences were also filtered using the "identify_chimeric_seqs.py" module of USEARCH.³⁰ The resulting reads with an average base quality Q score of <20 were discarded. The remainder of the steps (explained below) were performed with the Quantitative Insight into Microbial Ecology (QIIME) suite, version 1.8 and an in-house developed wrapper for QIIME called QWRAP.^{28,31–33} Following quality control filtering, quantification of rRNA copies was performed, and we

excluded any sample containing fewer than 10,000 passing reads. This allowed for filtration of extraction and reagent contaminants.

Sequences were grouped into operational taxonomic units (OTUs) using the clustering program UCLUST at a similarity threshold of 97%.³⁰ The Ribosomal Database Program (RDP) classifier was used to make taxonomic assignments (to the species level) for all OTUs at a confidence threshold of 80% (0.8). The RDP classifier was trained using a version of the Greengenes (v13_8) 16S rRNA database modified to include bacterial vaginosis associated bacteria (BVAB).^{33–35} Details of our BVAB prediction model have been previously described in detail.^{13,26} The resulting OTU table included all OTUs, their taxonomic identification, and abundance information. To account for differences in read depth across different samples, the OTUs were rarified at a common sequence depth. OTUs whose average abundance was less than 0.05% were filtered out. OTUs were then grouped together to summarize taxon abundance at different hierarchical levels of classification (e.g. phylum, class, order, family, genus). These taxonomy tables were also used to generate bar charts of taxon abundance. Multiple sequence alignment of OTUs was performed with PyNAST.²⁹

The number of unique observed species seen by sequences per sample was calculated. Alpha diversity (the diversity within a given sample) was evaluated using Shannon, Chao1, and Simpson diversity metrics as implemented in QIIME.³⁶ Clustering of microbial communities or between sample diversity (beta-diversity) was assessed by Bray-Curtis or Unifrac and visualized by principal coordinates analysis (PCoA).^{37,38}

Statistical Analysis

As per study design, patients were categorized into two groups: SPTB (n=20) or term birth (n=20) (total n=40). Descriptive characteristics of the study populations were reported using means, medians, ranges, and proportions. Differences in these demographic variables were analyzed with Wilcoxon rank sum, Chi-square, Fisher's exact test, or Student's t-test as appropriate. The Welch t-test was utilized to compare the differences in observed species as well as alpha-diversities (by Shannon, Chao1, and Simpson indices) between the SPTB and term groups. The difference in overall microbiome composition of detected microbial DNA between the SPTB and term groups was analyzed using permutation-based ANOVA over all three beta-diversity measures—Bray-Curtis, weighted UniFrac, and unweighted UniFrac—with statistical significance measured using PERMANOVA. Statistical significance for these measures was evaluated at a 0.05 level. Taxa specific analyses were conducted with the Kruskal-Wallis test. Given the multiple comparisons for taxa-specific analyses, false discovery rate (FDR) corrected p-values were also calculated.

RESULTS

All 40 samples met quality control measures, had detectable taxa, and were included for analysis. The 40 samples had a total of 3.58 million sequence reads corresponding to 894,779,750 base pairs of sequence – above the contaminant and control threshold. Demographic characteristics of the SPTB group (n=20) and term birth group (n=20) are presented in Table 1. Groups were similar with the exception of gestational age at birth–

which was expected as the result of the group definitions. For these 40 samples, the ten most common organisms noted in the overall sample population are presented in Table 2.

There were greater numbers of unique species observed in the serum of women with SPTB as compared to term birth ($p=0.046$) (Figure 1a). Similarly, there was a higher mean alpha-diversity (within sample diversity) by the Shannon index observed in the SPTB group as compared to the term group (6.44 vs. 5.39, $p = 0.024$) (Figure 1b), but not by Chao1 ($p=0.067$) or Simpson (0.052) indices (boxplots for Chao1 and Simpson not presented).

When analyzing differences in beta-diversity between the two groups by principal coordinates analysis, there were significant differences by Bray-Curtis clustering (PERMANOVA $p= 0.03$) in the serum microbiome of women with SPTB as compared to term birth (Figure 1c). While subclusters were noted (Figure 1c), no available demographic characteristics explained these unique clusters. This difference was not statistically significant when analyzing by the other measures of beta-diversity (weighted UniFrac PERMANOVA $p=0.13$; unweighted UniFrac PERMANOVA $p=0.11$; principal coordinates plots for weighted and unweighted UniFrac not presented).

Taxa level analyses at the phylum, class, order, family, and genus level were performed to assess for differences between the SPTB and term birth groups. Numerous taxa were significantly different between these groups ($p<0.05$) (Figure 2 is a stacked bar plot showing the taxa comparisons at the phylum level. Numerous taxa, specifically in the Firmicutes, Proteobacteria, and Actinobacteria phyla, had $>1\%$ abundance in the samples and were significantly different between groups ($p<0.05$)—these taxa are presented in Table 3. While these taxa were significantly different between groups in univariate analysis, it is important to note that none passed the false-discovery rate test.

COMMENT

We have demonstrated that there is microbial DNA detectable in the serum of pregnant women collected at 15–20 weeks gestational age. Furthermore, we have demonstrated higher microbial diversity as well as microbial clustering differences in the midtrimester maternal serum microbiome in women who go on to have a SPTB as compared to those who experience a term birth.

Since one of our objectives was to determine if microbial DNA could be detected in the midtrimester serum of pregnant women, our sample size is small and limits definitive conclusions regarding a specific serum microbiome as well as specific taxa differences between women who have a SPTB or term birth. However, we found preliminary evidence for significant differences in presence of microbial DNA that should be further explored in larger studies with quantitative PCR using primers specific for target microbes, since they may represent a potential method to identify women early in pregnancy who are at an increased risk of SPTB. Since blood sampling is a routine part of prenatal care, this could represent an easy way to identify at-risk women that may be candidates for targeted therapies or risk-reducing strategies.

Prior studies have shown microbial variations between women who experience PTB and term birth in a variety of maternal niches: cervicovaginal fluid, amniotic fluid, and placental tissue. Multiple studies have shown that, compared to women who deliver at term, women who deliver preterm have decreased cervicovaginal microbial diversity.^{8,12,18,20} Both Baldwin et al and Kacerovsky et al have described alterations in certain *Lactobacillus spp.* as well as the persistence of pathogenic species in vaginal samples of women who experience PROM.^{10,11} More recently, Elovitz et al described multiple significant taxa-specific differences in the cervicovaginal microbiome between women with a SPTB and those with term birth.¹⁷ Specifically, they described that *Bifidobacterium* species appear to be protective against SPTB, while *BVAB2*, *BVAB3*, and *Mobiluncus* were associated with an increased risk of SPTB (findings observed longitudinally at varying gestational ages between 16–28 weeks' gestation).¹⁷ Both molecular methods and traditional culture have also shown similar microbial disturbances in the amniotic fluid and placental tissue of women who experience SPTB.^{4,14,15, 39,40} While none of these studies have conclusively shown validated, predictable microbial signatures associated with SPTB (probably secondary to varied studied populations with respect to race/ethnicity and history of prior preterm birth, sampling timing, and sequencing methods [16S primers versus whole genome sequencing]), they emphasize the need to comprehensively investigate microbial changes as a causal mechanism in the SPTB pathway.

While the lower genital and intrauterine environments have begun to be examined, there is a paucity of data on microbial DNA changes in maternal blood. Previous investigators have shown the presence of systemic markers of inflammation in serum of women who have a SPTB – even as early as the midtrimester.^{4,41,42} Given that there are systemic signs of inflammation associated with SPTB early in pregnancy, it is plausible that the microbial DNA changes associated with PTB may also be reflected in maternal serum. However, traditional culture methods have limited our ability to investigate the existence of microbes in serum without the presence of overt infection or sepsis. Our study shows that there is detectable microbial DNA present in maternal serum – a finding previously shown in non-pregnant individuals by Nikkari et al.⁴³ Importantly, taxa that we isolated from serum specimens resemble taxa isolated from the oral, gut, and placental microbiomes (with some vaginal microbiome contribution) and not “skin” microbiome contamination from phlebotomy. Finding this DNA in maternal serum does not imply viable organisms—it may be free or from dead organisms. This may suggest a hematogenous spreading of low levels of organisms or at least microbial DNA in the blood that may originate from other maternal niches involved in the pathogenesis of SPTB. While it is premature to speculate on the presence of a serum microbiome, we have shown the presence of microbial DNA in the serum (whether from cell-free DNA, living or dead microorganisms). In addition, we have shown differences in midtrimester serum microbial DNA between women who have a SPTB and term birth. Thus, maternal serum microbial DNA might be able to be utilized to develop screening tools for SPTB.

Strengths of our study include the use of samples from a cohort of women followed at our institution with well-described outcome information. Furthermore, these samples had not been stored for a long duration (collected in 2014) and had never been subject to thawing previous to this study. Another strength of this study is the stratification into group extremes.

In other words, term births at 39–40 weeks were compared with early spontaneous preterm births (≤ 33 weeks). Given that the microbiome composition in the cervicovaginal compartment tends to be stable throughout pregnancy, especially in term births where minimal microbial variations have been described, no late preterm or early term birth samples were utilized in order to uncover a true “microbial genotype” associated with the outcome of interest. Similarly, by matching for race/ethnicity, we accounted for microbial differences introduced by that variable.

One limitation of this study is the small sample size. We had two objectives—first, to evaluate whether there was detectable microbial DNA in the maternal serum, and second, to evaluate whether there may be microbial DNA signatures associated with birth timing. While no specific taxa level differences passing the false discovery rate test were seen (although multiple differences prior to FDR test were noted), we did successfully detect microbial DNA in maternal serum (some microbes of which originate in the genital tract) and noted a statistically significant difference in the number of unique observed species, one alpha-diversity metric, and one beta-diversity metric between these two groups. Thus, while small in scope, this positive study provides rationale as well as parameters needed to calculate sample size for further larger studies to elaborate on our findings.

There are currently no universal primers for analysis of the lower genital tract or other low biomass environments. Each primer region that has been employed—V1V3, V4, V3V4, and V3V5—has unique advantages and disadvantages in identifying certain bacterial taxa (e.g. *Lactobacillus spp.*, *Gardnerella*). While whole genome sequencing does not utilize these primer regions and thus improves accuracy of species detection, it has its own limitations—namely, high cost and extensive bioinformatics computational needs. Therefore, 16S amplicon sequencing still is a widely used method. Our group and others have successfully used the V4 region – with our group using a reference database modified for the ability to detect bacteria specific to vaginal communities (BVABs) at a similar level to the use of alternative primers.^{13,26,27,44–46}

In summary, maternal serum contains microbial DNA, and microbial DNA differences associated with SPTB are detectable in maternal serum collected between 15–20 weeks gestation. If these findings are replicated in a larger population, this may represent a method to develop a screening test for patients at risk for SPTB. Specifically, these findings may be used to provide a foundation to develop a test requiring QPCR to detect specific microbial signatures. As all pregnant women routinely have blood drawn as part of prenatal care, maternal blood samples would be an easy milieu for PTB screening with rapid QPCR-based tests. Ultimately, at-risk patients could be identified and putative therapies for risk reduction could be evaluated in this group to determine their ability to decrease the morbidity and mortality associated with preterm birth.

Acknowledgements:

The following are acknowledged for their support of the Microbiome Resource at the University of Alabama at Birmingham: Comprehensive Cancer Center (P30AR050948), Center for AIDS Research (5P30AI027767), Center for Clinical Translational Science (UL1TR001417), University Wide Institutional Core and Hefflin Center for Genomic Sciences

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Figure 1.

Representations of differences in microbial composition between women who experienced spontaneous preterm birth (SPTB) and term birth by three analytic methods.

A) Rarefaction curve demonstrating the number of observed species seen by sequences per samples for the SPTB (blue) and term (red) groups. Greater number of unique species were detected in the preterm as compared to term samples ($p=0.046$).

B) Box plot of Shannon indices for the term birth group (Left bar; mean Shannon index = 5.39) and SPTB group (Right bar; mean = 6.44). The groups were significantly different ($p = 0.024$).

C) Comparison of the overall microbial diversity between serum samples from patients with term versus SPTB. Principal coordinate analysis was used to generate a plot using Bray-Curtis metrics. Clustering of samples in the two groups (red = term birth, blue = SPTB) was significantly different ($p<0.03$).

Figure 2.

Phylum level relative abundance (y-axis) in samples comparing between women having spontaneous preterm birth (SPTB) and term birth

Table 1.

Baseline characteristics of sample population

	SPTB (n=20)	Term birth (n=20)	p-value
Maternal age (y)	22.9 ± 2.7	22.7 ± 4.3	0.86
Race			
Black	17 (85.0%)	17 (85.0%)	>0.99
White	3 (15.0%)	3 (15.0%)	
Payor status			
Government	14 (70.0%)	14 (70.0%)	0.37
Indigent	1 (5.0%)	4 (20.0%)	
Private	3 (15.0%)	2 (10.0%)	
Unknown	2 (10.0%)	0 (0%)	
Marital Status			
Single	40 (100.0%)	39 (95.0%)	>0.99
Married	0 (0.0%)	1 (5.0%)	
Body mass index (kg/m ²)	28.0 ± 9.8	28.2 ± 8.6	0.95
Smoking	5 (25.0%)	4 (22.2%)	>0.99
Birth gestational age	29.0 (25.8–30.8)	39.6 (39.3–41.1)	<0.0001

SPTB = spontaneous preterm birth

Data are presented as n (%), mean ± standard deviation, or median (interquartile range).

Table 2.

Most common ten organisms present in maternal serum microbiome

	Taxa	Average Relative Abundance (%)
1	p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__Tissierella_Soehngenia; s__	11.8%
2	p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__	4.2%
3	p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae	3.8%
4	p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Escherichia; s__coli	3.0%
5	p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Stenotrophomonas; s__	2.8%
6	p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium	2.7%
7	p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Enterococcaceae; g__Enterococcus; s__	2.1%
8	p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__	2.0%
9	p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus	1.7%
10	p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus	1.6%

Table 3.

Taxa at each taxonomic rank (level) that were significantly different between the spontaneous preterm birth (SPTB) and term birth groups ($p < 0.05$) but did not pass the false-discovery rate test (FDR p-values presented). Bolded rows denote taxa that had mean relative abundance $> 1\%$ in either the SPTB or term groups or both.

Level	Operational Taxonomic Unit (OTU)	FDR p-value
L2	k_Bacteria;p_Actinobacteria	0.43
L2	k_Bacteria;p_Cyanobacteria	0.47
L3	k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria	0.57
L3	k_Bacteria;p_Actinobacteria;c_Actinobacteria	0.57
L3	k_Bacteria;p_Cyanobacteria;c_Synechococcophycideae	0.57
L4	k_Bacteria;p_Cyanobacteria;c_Synechococcophycideae;o_Pseudanabaenales	0.77
L4	k_Bacteria;p_Tenericutes;c_Mollicutes;o_Anaeroplasmatales	0.77
L5	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae	0.57
L5	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae	0.57
L5	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Barnesiellaceae]	0.57
L5	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Micrococcaceae	0.57
L5	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae	0.57
L5	k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Paenibacillaceae	0.57
L5	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Alteromonadales;f_Shewanellaceae	0.57
L5	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Geodermatophilaceae	0.57
L5	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Xanthobacteraceae	0.57
L5	k_Bacteria;p_Cyanobacteria;c_Synechococcophycideae;o_Pseudanabaenales;f_Pseudanabaenaceae	0.57
L5	k_Bacteria;p_Tenericutes;c_Mollicutes;o_Anaeroplasmatales;f_Anaeroplasmataceae	0.57
L5	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Burkholderiaceae	0.57
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Acidaminococcus	0.23
L6	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Comamonadaceae;g_Limnobacter	0.23
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Faecalibacterium	0.23
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Roseburia	0.23
L6	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae;g_Streptococcus	0.23
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Dialister	0.23
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Lachnospira	0.23
L6	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Alteromonadales;f_[Chromatiaceae];Other	0.38

Level	Operational Taxonomic Unit (OTU)	FDR p-value
L6	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Geodermatophilaceae;g_	0.38
L6	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacteriales;f_Caulobacteraceae;g_Brevundimonas	0.38
L6	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidiales;f_Porphyromonadaceae;g_Porphyrimonas	0.38
L6	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae;g_Escherichia	0.38
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Coproccoccus	0.38
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Phascollartobacterium	0.38
L6	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Actinomycetaceae;g_Actinomycetes	0.38
L6	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Sutterella	0.38
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Epulopectum	0.38
L6	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidiales;f_Barnesiellaceae;g_	0.38
L6	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Micrococccaceae;g_Rothia	0.38
L6	k_Bacteria;p_Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales;f_Flavobacteriaceae;g_Flavobacterium	0.38
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Blautia	0.38
L6	k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Paenibacillaceae;g_Paenibacillus	0.38
L6	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Burkholderiaceae;g_Salnispora	0.38
L6	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Ruminococcus	0.38
L6	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Alteromonadales;f_Shewanellaceae;g_Shewanella	0.41
L6	k_Bacteria;p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_Collinsella	0.42
L6	k_Bacteria;p_Thermi;c_Deinococci;o_Thermales;f_Thermaceae;g_Thermus	0.42
L6	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Xanthobacteraceae;g_Azorhizobium	0.42
L6	k_Bacteria;p_Cyanobacteria;c_Synechococcophycideae;o_Pseudanabaenales;f_Pseudanabaenaceae;g_Leptolyngbya	0.42
L6	k_Bacteria;p_Tenericutes;c_Mollicutes;o_Anaeroplasmatales;f_Anaeroplasmataceae;g_Anaeroplasma	0.42
L6	k_Bacteria;p_Proteobacteria;c_Epsilonproteobacteria;o_Campylobacteriales;f_Helicobacteraceae;g_Sulfurimonas	0.42
L6	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae;g_Alkanindiges	0.44
L6	k_Bacteria;p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Catenibacterium	0.44
L6	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Aerococcaceae;g_Alloiococcus	0.45