Dental caries as a risk factor for bacterial blood stream infection (BSI) in children undergoing hematopoietic cell transplantation (HCT)

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Background. Hematopoietic cell transplantation (HCT) is a potentially curative therapy for a wide range of pediatric malignant and nonmalignant diseases. However, complications, including blood stream infection (BSI) remain a major cause of morbidity and mortality. While certain bacteria that are abundant in the oral microbiome, such as *S. mitis*, can cause BSI, the role of the oral microbial community in the etiology of BSI is not well understood. The finding that the use of xylitol wipes, which specifically targets the cariogenic bacteria *S. mutans* is associated with reduced BSI in pediatric patients, lead us to investigate dental caries as a risk factor for BSI. **Methods.** A total of 41 pediatric patients admitted for allogenic or autologous HCT, age 8 months to 25 years, were enrolled. Subjects with high dental caries risk were identified as those who had dental restorations completed within 2 months of admission for transplant, or who had untreated decay. Fisher's exact test was used to determine if there was a significant association between caries risk and BSI. Dental plaque and saliva were collected on a cotton swab from a subset of 4 high caries risk (HCR) and 4 low caries risk (LCR) children following pretransplant conditioning. 16SrRNA sequencing was used to compare the microbiome of HCR and LCR subjects and to identify microbes that were significantly different between the 2 groups. **Results.** There was a statistically significant association between caries risk and BSI (p<0.035) (Fisher's exact test). Multivariate logistic regression analysis showed children in the high dental caries risk group were 21.39 times more likely to have BSI, with no significant effect of age or mucositis severity. HCR subjects showed significantly reduced microbial alpha diversity as compared to LCR subjects. LEfse metagenomic analyses, showed the oral microbiome in HCR children enriched in order Lactobacillales. This order includes *Streptococcus* and *Lactobacillus,* both which contain bacteria primarily

associated with dental caries. **Discussion.** These findings support the possibility that the cariogenic microbiome can enhance the risk of BSI in pediatric populations. Future metagenomic analyses to measure microbial differences at, before, and after conditioning related to caries risk, may further unravel the complex relationship between the oral microbiome, and whether it affects health outcomes such as BSI.

1 **Dental caries as a risk factor for bacterial blood stream infection (BSI) in children**
2 **undergoing hematopoietic cell transplantation (HCT)**

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48 **Abstract**
49 **Background.** Hematopoietic cell transplantation (HCT) is a potentially curative therapy for a wide
50 range of pediatric malignant and nonmalignant diseases. However, complications, including blood
51 stream infection (BSI) remain a major cause of morbidity and mortality. While certain bacteria
52 that are abundant in the oral microbiome, such as *S. mitis*, can cause BSI, the role of the oral
53 microbial community in the etiology of BSI is not well understood. The finding that the use of
54 xylitol wipes, which targets the cariogenic bacteria *S. mutans* is associated with reduced BSI in
55 pediatric patients, lead us to investigate dental caries as a risk factor for BSI.
56 **Methods.** A total of 41 pediatric patients admitted for allogenic or autologous HCT, age 8 months
57 to 25 years, were enrolled. Subjects with high dental caries risk (HCR) were identified by
58 reviewing dental clearance records to identify subjects as those who had dental restorations
59 completed within 2 months of admission for transplant, or who had untreated decay. The
60 remaining subjects were classified as low dental caries risk (LCR). Fisher's exact test was used
61 to determine if there was a significant association between caries risk and BSI. Dental plaque
62 and saliva were collected on a cotton swab from a subset of 4 HCR and 4 LCR children following
63 pretransplant conditioning. 16SrRNA sequencing was used to compare the microbiome of HCR
64 and LCR subjects and to identify microbes that were significantly different between the 2 groups.
65 **Results.** There was a statistically significant association between caries risk and BSI ($p<0.035$)
66 (Fisher's exact test). Multivariate logistic regression analysis showed children in the high dental
67 caries risk group were 21.39 times more likely to have BSI, with no significant effect of age or
68 mucositis severity. HCR subjects showed significantly reduced microbial alpha diversity as
69 compared to LCR subjects. LEfse metagenomic analyses, showed the oral microbiome in HCR
70 children enriched in order Lactobacillales. This order includes *Streptococcus* and *Lactobacillus,*
71 both which contain bacteria primarily associated with dental caries.
72 **Discussion.** These findings support the possibility that the cariogenic microbiome can enhance
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74 differences at, before, and after conditioning related to caries risk, may further unravel the
75 complex relationship between the oral microbiome, and whether it affects health outcomes such
76 as BSI.

Introduction

Hematopoietic cell transplantation (HCT) is a potentially curative therapy for a wide range of pediatric malignant and nonmalignant diseases. However, complications associated with HCT conditioning regimens and post-HCT immunocompromised status, such as mucositis and blood stream infection (BSI), remain a major cause of morbidity and mortality (Dahllof 2008; McNamara et al. 2018). The incidence of BSI is reported to vary from 22% to 55.8% and is related to prolonged neutropenia, mucosal damage, and extensive use of venous central lines (Gustinetti & Mikulska 2016).

In this study we tested the hypothesis that dental caries contributes to BSI risk in pediatric HCT recipients. This possibility is supported by the study by Badia et al. (Badia et al. 2020) , which showed daily use of xylitol wipes in children undergoing HCT significantly reduced dental plaque, mucositis and bacteria known to cause BSI. Xylitol reduces dental plaque by substituting for carbohydrates consumed by the acid and extracellular polysaccharide producing cariogenic bacteria, *S mutans* (Söderling & Pienihäkkinen 2020). Reduced extracellular polysaccharides means that plaque is less adherent and more easily removed from the tooth surface (Söderling & Pienihäkkinen 2020). This suggests that changes in cariogenic bacteria contributes to BSI risk.

To address a possible role of a cariogenic microbiome as a risk factor for BSI, we enrolled 41 pediatric allogenic or autologous HCT recipients, age 8 months to 25 years, and classified them as either high caries risk (HCR) or low caries risk (LCR) based on pretransplant dental clearance records. We used 16SrRNA sequencing to compare the plaque/saliva microbial composition in a subgroup of 4 HCR and 4 LCR subjects.

Materials & Methods

Subject enrollment and sample collection:

Study patients: The University of California San Francisco granted approval to carry out this study within its facilities under IRB protocol # 15-18297. Forty-one of 43 pediatric HCT recipients, age 8 months to 25 years, treated in the University of California, San Francisco (UCSF) Benioff Children's Hospital and approached for participation in the study, were enrolled between the years 2016 and 2019. Inclusion criteria were HCT recipients who could cooperate with study procedures, and parents and patients willing to participate and sign informed consent and assent forms. Exclusion criteria were patients or parents unable to understand or participate in study procedures, or allergies to multiple hygiene and cosmetic products. Written consents by parents and assent by children were obtained for all subjects.

Dental caries risk was determined by retrospective examination of dental clearance records. Past caries experience is a robust predictor of the disease (Philip et al. 2018), and we classified participants with dental clearances stating "clear/no caries detected/minimal decay", or extractions of third molars with no other mention of dental caries or restorations, as low caries risk (LCR). Subjects with dental clearances stating "treatment for multiple dental caries" or indications of untreated decay 2 months prior to admission for transplant, were classified as high caries risk (HCR),

All patients were administered prophylactic levofloxacin from Day -2 until neutrophil recovery (Alexander et al. 2018). Mucositis was assessed from the medical records with daily assessments by nurses and physicians recorded according to the WHO classification standards. Bacterial blood stream infections (BSI) were defined as any positive bacterial culture of (central line) blood samples during the transplantation hospital stay.

126 Oral samples were collected by using cotton swab to collect saliva and plaque by rubbing the
127 cotton swab across the tooth surfaces and under the tongue. All samples were stored at -80⁰C
128 until they were submitted for 16sRNA sequencing.
129
130 16S rRNA sequencing and bioinformatics
131 Oral samples were taken from the entire cohort and sent to uBiome for sequencing. We got back
132 fastq files for 31 samples from uBiome.
133 Samples were processed and sequenced by uBiome (San Francisco, CA) as reported by
134 Almonacid et al. (Almonacid et al. 2017). Briefly, the V4 variable region of the 16S rRNA genes
135 was amplified by PCR using universal primers (515F: GTGCCAGCMGCCGCGGTAA and 806R:
136 GGACTACHVGGGTWTCTAAT). The primers contained Illumina tags and barcodes. PCR
137 products were pooled, column-purified, and size-selected through microfluidic DNA fractionation.
138 Sequencing was performed in a pair-end modality on the Illumina NextSeq 500 platform rendering
139 2 x 150 bp pair-end sequences. The sequences obtained from uBiome included primers within
140 the 150 bp sequence, and when the primers were removed, the remaining sequences were up to
141 125 bp in length. With this length of sequence, there were not overlapping sequences greater
142 than 10 base pair, which is not ideal and therefore lead us to use only forward reads for our
143 analysis.
144
145 Bioinformatic analyses were done using QIIME2(version 2021.4) and R programming language
146 (version 4.1.2), on forward reads of the sequencing data. Reads containing more than 2 expected
147 errors and more than 8 consecutive same nucleotide repeats were discarded, and the raw
148 sequences were then truncated to 125pb, denoised, chimera filtered, clustered into sequence
149 variants. The taxonomy was assigned to operational taxonomic units (OTUs; 97% clustering)
150 using the HOMD (Human Oral Microbiome Database version15.2) database with QIIME2 pipeline
151 default settings. After analyzing rarefaction curves and the sampling depth that would retain the
152 most feature in QIIME pipeline we have left 8 samples for analyses. Feature table, rooted tree
153 and taxonomy files were then imported into R for diversity, PCoA and differential expression
154 analysis. Phyloseq package (version 1.30.0) was used for diversity and PCOA analyses. We
155 further evaluated the differentially expressed taxa with The Linear Discriminative Analysis (LDA)
156 Effect Size (LEfSe) program of the Galaxy environment".
157
158 Statistical analysis
159 Analysis of BSI as the dependent or the outcome variable of interest, was constructed as a
160 'yes/no', and was compared in the 2 groups using a Fisher's Exact test. A correlation matrix was
161 done comparing BSI, caries risk, age, gender, HCT-type (autologous or allogenic), cell type
162 (peripheral or bone marrow), length of time for mucositis, and highest mucositis grade. A multiple
163 regression analysis was done to further investigate the effect of age and mucositis, on caries risk
164 and BSI. The specific logistic regression model fitted to the data was: $Logit(BSI) = b_0 + b_1X_1 +$
165 $b_2X_2 + b_3X_3.$ All data analyses were conducted using R version 4.1.2. Effect Size (LEfSe) was
166 performed using the Galaxy web application using default settings. Statistical significance was
167 defined as $p \leq 0.05$.
168
169 **Results**
170 *Demographics*
171 In our study group, 27 of 41 subjects (65.9%) were classified as LCR and 34.1% were HCR. There
172 were no significant differences (p<0.05) between the groups in relative to age, gender, diagnosis,
173 stem cell source, or maximum mucositis grade (Table 1).

174
175 *Incidence of BSI*
176 All BSI incidences were between post-HCT and prior to ANC (absolute neutrophil count)
177 recovery/engraftment. Among them 57% were allogeneic HCT recipients (see Table 2). High
178 caries risk was significantly associated with subsequent BSI during transplantation (p=0.035)
179 (Figure 1)**.** Correlation analysis of other possible risk factors for BSI, showed only BSI to be
180 significantly positively correlated with caries risk, (p<0.05) (Figure 2). Multiple regression analysis
181 of the association of BSI with age, caries risk, and mucositis grade, showed an even more
182 significant association between BSI and caries risk (p=0.02) (Table 3).
183
184 *Microbiome analysis:*
185 We compared the oral microbiome of a combination of saliva and plaque collected from 4 HCR
186 and 4 LCR subjects following pretransplant conditioning (see Table 4). 16S rRNA sequencing
187 yielded mean number of 88,610.875 reads per sample (range 40,520.0-187,555.0) after quality
188 control processes. Raw sequencing files were deposited in GEO with submission number:
189 GSE199991. Clustering was carried out at 97% identity to yield 478 distinct OTUs with a length
190 of 125 bp. The HOMD framework allowed 98% of the OTUs to be assigned to uniquely identifiable
191 genus-level groups. 454 OTUs classified as bacteria remained after filtering, and were assigned
192 as Eukaryotes, Archaea, chloroplasts, and mitochondria. The most abundant phylum, family, and
193 genus, across all samples at >1% abundance, are shown in Figure 3, and summarized in Table
194 5, which shows the most abundant top four taxa across all samples.
195
196 Following conditioning, the alpha diversity in the LCR group was significantly greater than in the
197 HCR low caries risk group (p<0.05) (Figure 4a). Beta diversity metrics showed no clear clustering
198 by community composition by principal coordinate analysis (Figure 4 b, c, d).
199
200 We next performed LEfSe and DESeq2 analyses to identify individual microbial taxa that differed
201 between groups. Both LEfSe and DESeq2 were performed from phylum to species level (Figure
202 S.2). For the comparison HCR vs LCR, in DESeq2 analysis, 22 taxa were enriched in the LCR
203 group, while 8 taxa were enriched in the HCR group (Figure S.2). LEfSe showed 56 taxa enriched
204 in the LCR group while 2 taxa were enriched in HCR group (Figure 5, Figure S.3,4). Fifteen taxa
205 were enriched in the LCR group, order Lactobacillales and class Bacilli were the taxa significantly
206 enriched in HCR caries group in both analysis (Table 6).
207
208 While differences were only significant at the order and class level, relative abundances of
209 Streptococcus and Lactobacillus at the genus level showed relatively high levels of Streptococcus
210 in 3 of the 4 HCR subjects, and Lactobacillus in the 4th HCR subject. Genus Streptococcus and
211 Lactobacillus were at lower levels or undetectable in the LCR subjects (see Figure 6).
212
213 **Discussion**
214 In this study, we show a significant association between BSI in pediatric HCT recipients and caries
215 risk. Other studies report age (Akinboyo et al. 2020) and severity of mucositis (Gudiol et al. 2014;
216 Ustun et al. 2019) as associated with BSI, but in our study groups, these associations no longer
217 remained when dental caries risk was included in a multiple regression analysis. Similarly, our
218 correlation analysis showed only caries risk as associated with BSI. These findings indicate that
219 the oral microbiome associated with caries risk is a significant risk factor for BSI in pediatric HCT
220 patients.
221
222 The placement of dental restorations and extractions causes a transient bacteremia (Bahrani-
223 Mougeot et al. 2008), so while it is possible that increased BSI was related to bacteremia resulting
224 from dental restorations prior to transplant, such transient bacteremia would have most likely

225 resolved by the time of transplant. The placement of dental restorations does not reduce the
226 cariogenic bacterial load in the mouth (Philip et al. 2018); that must be done through other
227 approaches that target cariogenic bacteria.
228
229 We found no difference in beta diversity, but significantly decreased alpha diversity in the post-
230 conditioning HCR subgroup, as compared to the LCR subgroup. Beta diversity compares
231 microbial communities, whereas alpha diversity, also known as species diversity, compares the
232 diversity of species within a community. While we did not compare microbial diversity pre
233 conditioning, dental caries is not generally associated with changes in alpha diversity in the
234 pediatric oral microbiome (Jiang et al. 2016). Conditioning regimens, which use chemotherapy
235 and/or radiation can induce shifts in the oral microbiota (Hong et al. 2019; Laheij et al. 2019;
236 Vasconcelos et al. 2016). Therefore, reduced alpha diversity in the HCR oral microbiome post
237 conditioning suggests the possibility that the cariogenic microbiome has a unique ecological shift
238 induced by conditioning regimens.   Lower oral microbiome diversity at preconditioning of
239 autologous HCT patients has been found to be associated with a higher risk of relapse and worse
240 survival (de Molla et al. 2021). Therefore, reduced oral microbiome diversity in HCR patients
241 could also potentially impact long term transplant outcomes.

242 The HCR microbiome was significantly enriched in Lactobacillales. The order Lactobacillales
243 contains viridans group streptococci, including *S. mutans* and *S. sobrinus,* and also lactobacilli,
244 all of which are associated with dental caries (Beighton 2005), but their mechanistic links to
245 pediatric HCT morbidities remain unknown. The validity of our use of a retrospective assessment
246 of pretransplant dental clearance records to assess caries risk, was supported by the finding that
247 the relative abundance of bacteria from genus Streptococcus or Lactobacilli was enhanced in the
248 HCR as compared to LCR subjects.

249 Xylitol, which was previously found to be associated with a reduced incidence of BSI in pediatric
250 HCT recipients (Badia et al. 2020), targets the cariogenic bacteria, *S. mutans. S. mutans* does
251 not act alone in the development of dental caries, but it alters the local environment by forming a
252 favorable niche for other acidogenic and aciduric species to thrive (Lemos et al. 2019). Further
253 studies using metagenomic sequencing will identify unique features of the cariogenic microbiome
254 in HCT recipients; how it is altered by conditioning; and its association with other microbial niches,
255 such as the oral mucosa where the BSI-associated *S. mitis* (Doern & Burnham 2010) resides.
256
Conclusions
258 Much is to yet to be understood about the oral microbiome of children with undergoing HCT; how
259 changes in the oral microbiome, such as those that result in dental decay, affect the oral
260 environment following conditioning and transplant; and how changes in the oral microbiome may
261 affect BSI. The significant association between dental caries risk and BSI in our study population
262 suggests an important new consideration in pediatric transplant patients.
263

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Figure 1

The number of subjects with BSI in the high caries risk as compared to low caries risk

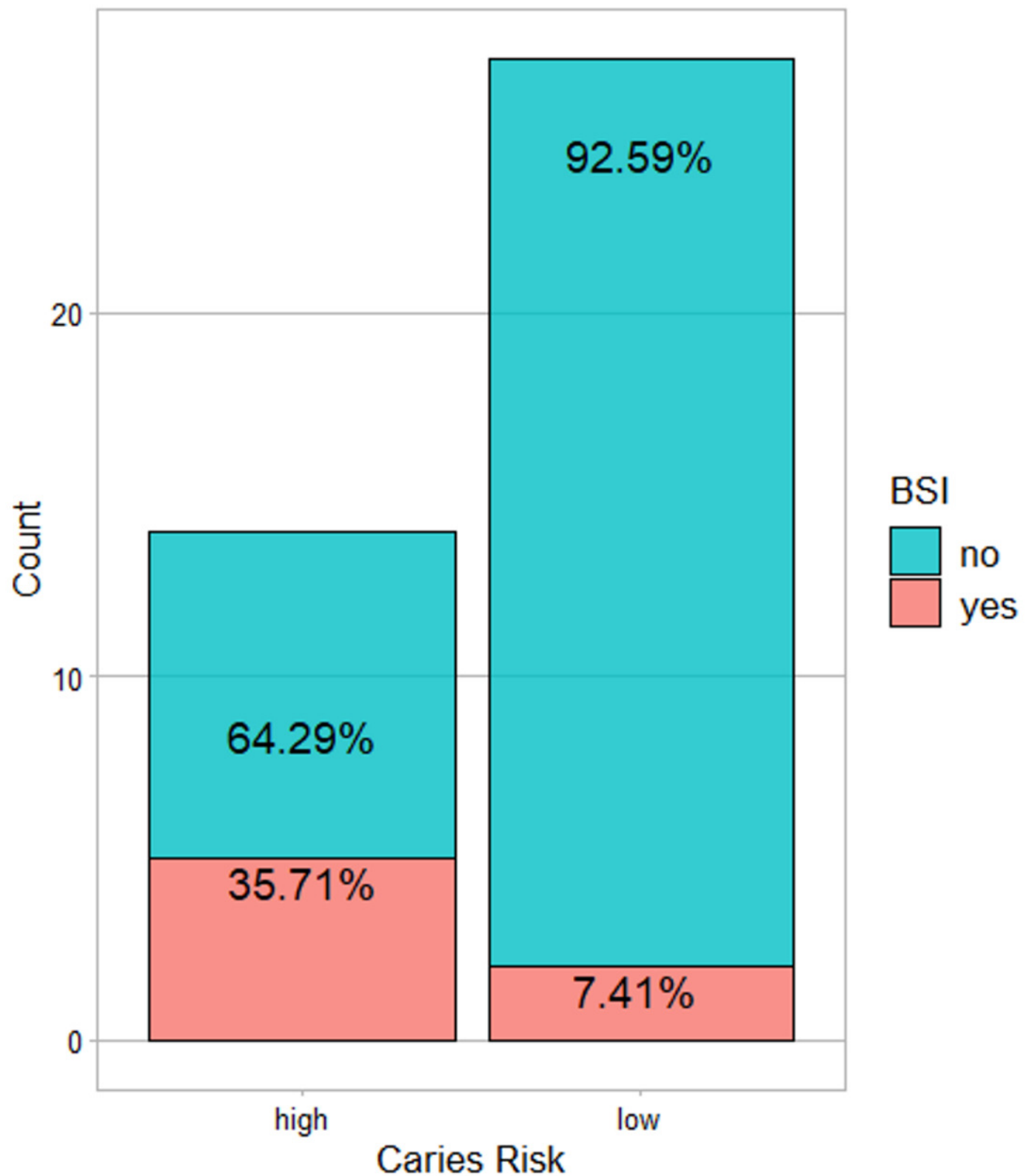



Figure 2

Correlation matrix plot showing the correlation between each pair of variables

A dot-representation was used where blue represents positive correlation and red negative. The larger the dot the larger the correlation. BSI showed correlations with high caries risk, age, and mucositis grade. BSI: blood stream infection. High caries risk was coded as 1, low caries risk as 0; Gender: male coded as 1, female coded as 0; HSCT-type: hematopoietic stem cell transplant type, auto type coded as 1, allo type coded as 0; Cell-type: PBSC coded as 1, BM coded as 0. Non-significant associations are crossed out

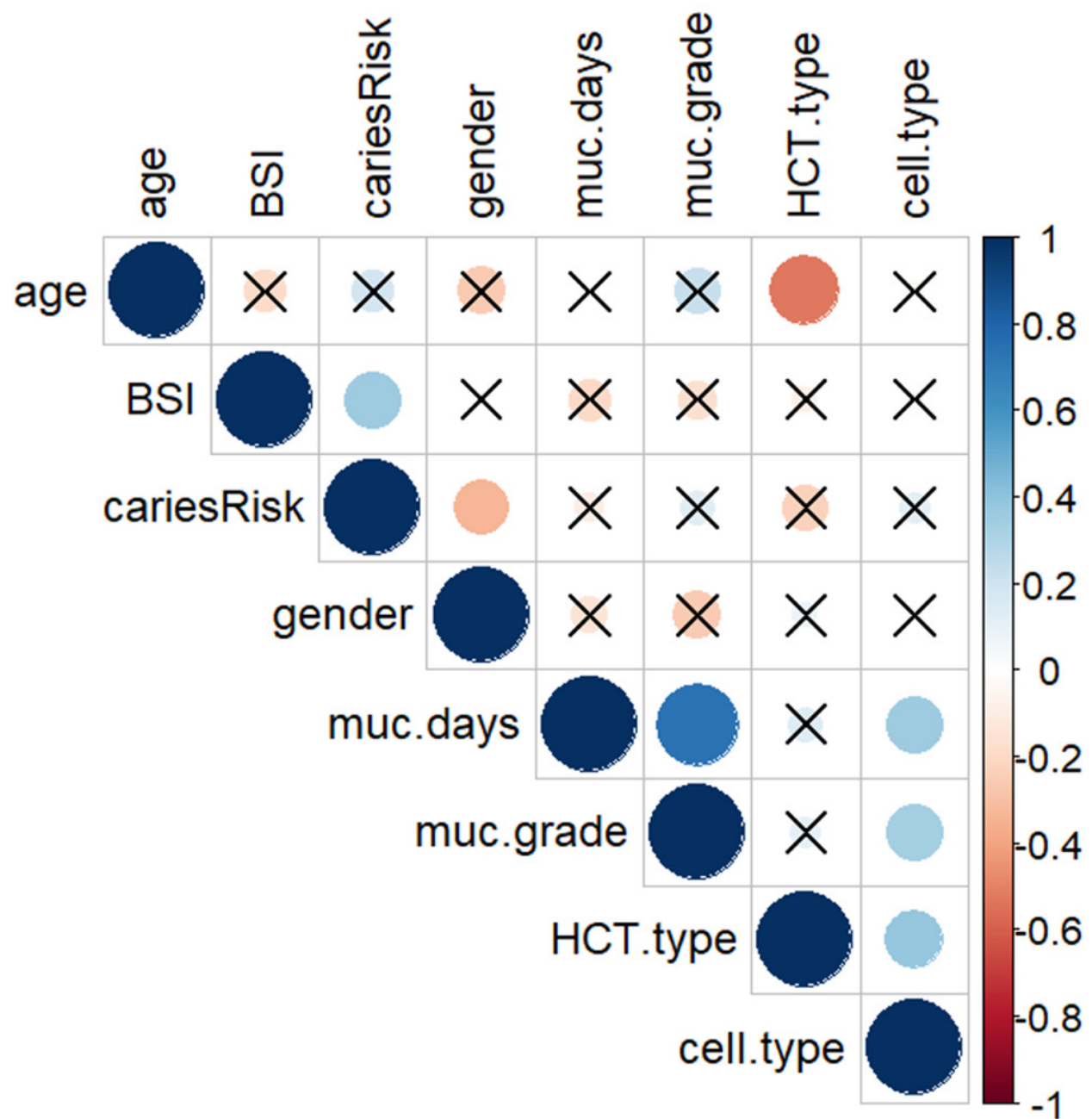



Figure 3

Bar plots showing the composition of bacterial communities by caries risk at a) phylum,
b) class, c) family, and d) genus level across all samples at > 1% abundance

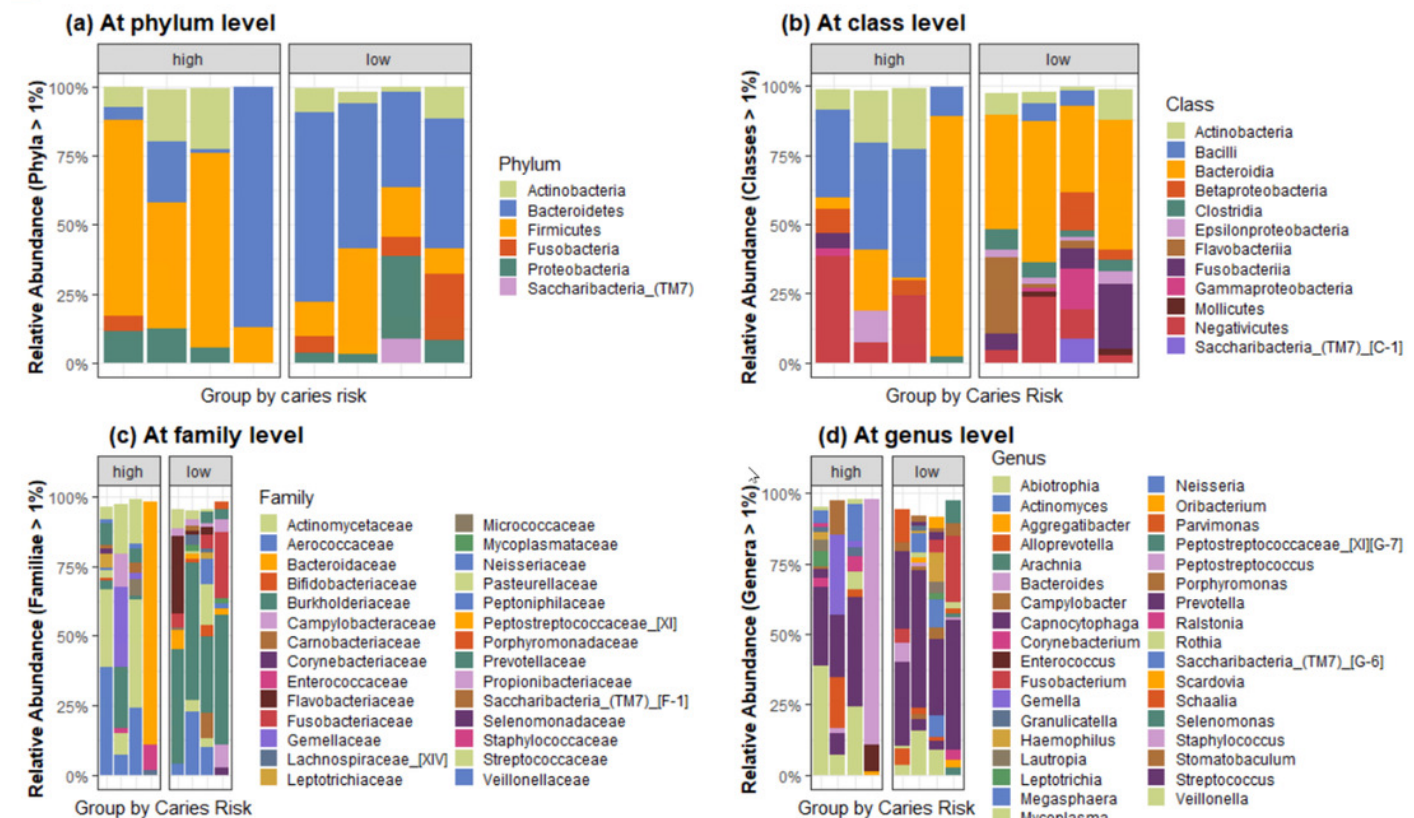



Figure 4

Diversity metrics and principal coordinate analysis

(a) Alpha diversity metric showing the difference in richness and evenness of communities.

Beta diversity metrics showing the clustering by community composition by principal

coordinate analysis using (b) Bray Curtis, (c) Unweighted UniFrac and (d) Weighted UniFrac

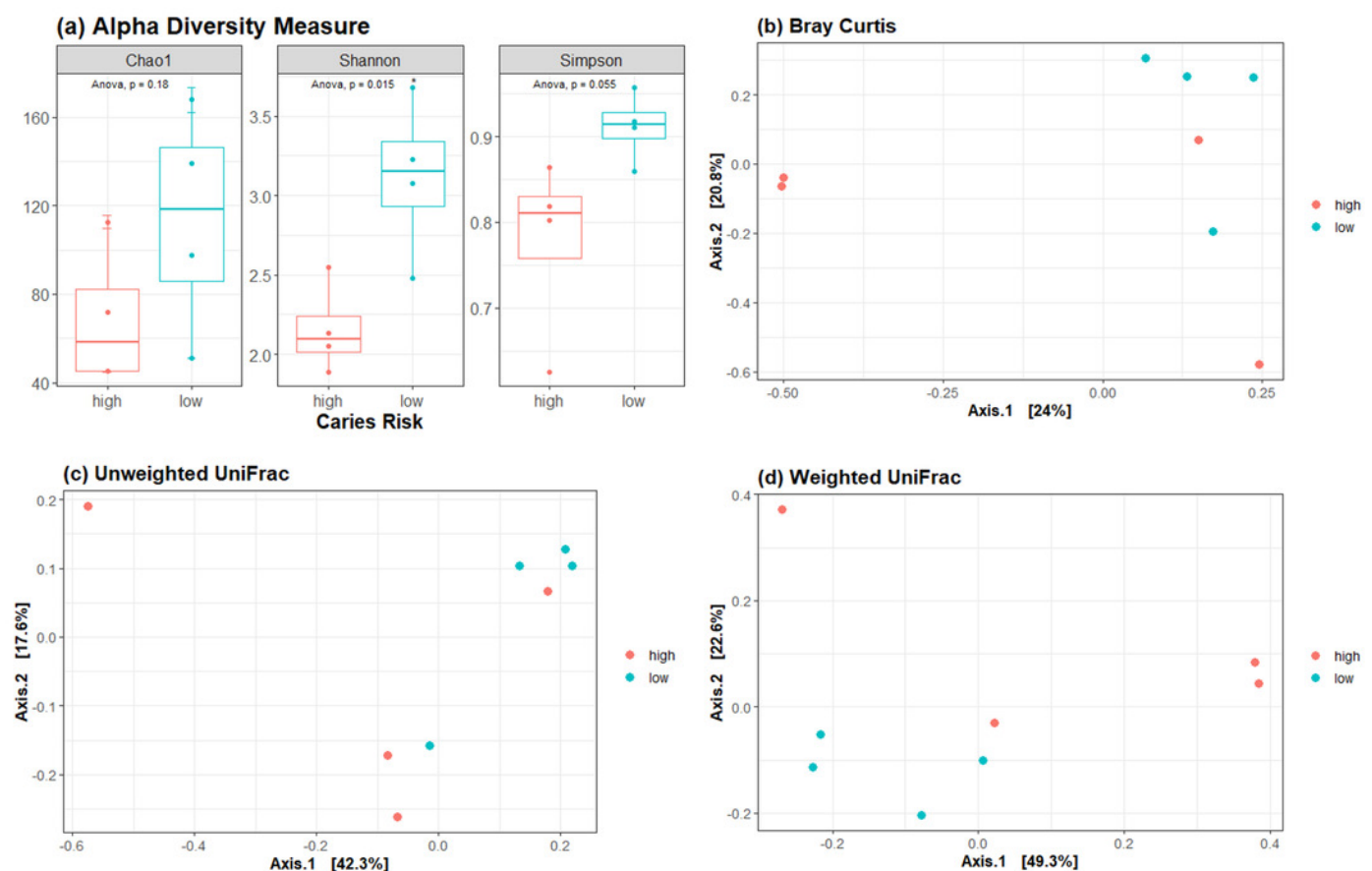
methods.

peerj-71564-BMT_Paper_Tables 7-19-2022

Table 1. Demographics of study population (n=41)

	Low caries risk (n, %)	High caries risk (n, %)	p -value
Subject number	27	14	
Gender (female)	8 (29.6)	9 (64.3)	0.072
Age (year, mean (range))	9.6 (0.7 - 25)	12.6 (1.6 - 21)	0.239
Diagnosis			0.439
Leukemia	7 (25.9)	7 (50.0)	
Lymphoma	2 (7.4)	1 (7.1)	
Non-malignant condition*	4 (14.8)	2 (14.3)	
solid Tumor	14 (51.9)	4 (28.6)	
HCT type			0.271
Allogenic	11 (40.7)	9 (64.3)	
Autologous	16 (59.3)	5 (35.7)	
Stem cell source			0.835
Bone marrow	4 (14.8)	1 (7.1)	
PBSC	23 (85.2)	13 (92.9)	
Mucositis grade 0-4** (mean (SD))	2.1 (1.4)	2.4 (1.2)	0.424

1   *Non-malignant condition: Severe aplastic anemia, Severe congenital neutropenia, and Primary
2   immunodeficiency disorder.
3   ** WHO grade ●Grade 0 (none)– No oral mucositis
4   ●Grade 1 (mild)– Erythema and soreness
5   ●Grade 2 (moderate)– Ulcers; able to eat solid food
6   ●Grade 3 (severe)– Ulcers; but requires liquid diet (due to mucositis)
7   ●Grade 4 (life-threatening) – Ulcers; alimentation not possible due to mucositis
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Table 2. Demographics of BSI patients (n=6)

	Low caries risk (n%)	High caries risk (n%)	p -value
Subject number	2	4	
Gender (female)	1 (50.0)	2 (50.0)	1.000
Age (year, mean (range))	1.6 (1.3-2)	8.2 (1.6-19)	0.308
Diagnosis			0.223
Leukemia	0 (0.0)	2 (50.0)	
Non-malignant condition*	0 (0.0)	1 (25.0)	
Solid Tumor	2 (100.0)	1 (25.0)	
HCT type			0.386
Allogenic	0 (0.0)	3 (75.0)	
Autologous	2 (100.0)	1 (25.0)	
Stem cell source			1.000
BM	0 (0.0)	1 (25.0)	
PBSC	2 (100.0)	3 (75.0)	
Mucositis grade			0.223
Grade 0	1 (50.0)	0 (0.0)	
Grade 1-2	0 (0.0)	2 (50.0)	
Grade 3	1 (50.0)	2 (50.0)	

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Table 3: Association with BSI

			BSI	
Predictors	*Estimate*	*Odds Ratios*	*95% Confidence Interval*	*p-value*
(Intercept)	-0.959	0.38	0.05 – 2.09	0.301
Age	-0.130	0.88	0.72 – 1.02	0.124
Caries risk	3.063	21.39	2.44 – 427.55	**0.016**
Mucositis grade	-0.487	0.61	0.25 – 1.32	0.225
Observations	*41*			
R^2 Tjur	*0.27*			

Table 4: Demographics of subjects for microbiome analyses

	Low caries risk (n, %)	High caries risk (n, %)	p -value
Subject number	4	4	
Gender (female)	1 (25.0)	3 (75.0)	0.48
Age (year, mean (range))	16.0 (12-21)	13.1 (5.4 - 21)	0.5
Diagnosis			0.41
Leukemia	2 (50.0)	4 (100.0)	
Lymphoma	2 (50.0)	0 (0.0)	
HCT type			1.0
Allogenic	3 (75.0)	4 (100.0)	
Autologous	1 (25.0)	0 (0.0)	
Stem cell source			
PBSC	4 (100)	4 (100)	
Mucositis grade			0.48
Grade 1-2	1 (25.0)	3 (75.0)	
Grade 3-4	3 (75.0)	1 (25.0)	

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Table 5 Most abundant phylum, Class, Family, and Genus

At Phylum level	At Class level	At the Family level	At the Genus level
Bacteroidetes (44.48%)	Bacteroidia (36.60%)	Prevotellaceae (27.88%)	Prevotella (24.83%),
Firmicutes (27.96%)	Negativicutes (12.16%)	Veillonellaceae (11.76%)	Veillonella (11.03%)
Proteobacteria (11.88%)	Bacilli (11.90%)	Flavobacteriaceae (8.44%)	Capnocytophaga (8.55%)
Actinobacteria (7.56%)	Flavobacteriia (8.25%)	Bacteroidaceae (8.23%)	Bacteroides (8.39%)

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Table 6 significantly different taxa in LCR as compared to HCR in both analysis (DESeq2, LEfSe**)**

log2FoldChange	Taxa
-4.813	p_Firmicutes,c_Bacilli,o_Lactobacillales
-6.16817	p_Firmicutes,c_Bacilli
9.734868	p_Bacteroidetes,c_Bacteroidia,o_Bacteroidales,f_Prevotellaceae,g_Prevotella,s_nigrescens
11.36474	p_Fusobacteria,c_Fusobacteriia,o_Fusobacteriales,f_Fusobacteriaceae,g_Fusobacterium,s_sp._HMT_203
8.819969	p_Firmicutes,c_Negativicutes,o_Veillonellales,f_Veillonellaceae,g_Megasphaera,s_micronuciformis
10.11979	p_Bacteroidetes,c_Bacteroidia,o_Bacteroidales,f_Prevotellaceae,g_Prevotella,s_marshii
10.46362	p_Firmicutes,c_Mollicutes,o_Mycoplasmatales,f_Mycoplasmataceae,g_Mycoplasma
8.713728	p_Firmicutes,c_Negativicutes,o_Veillonellales,f_Veillonellaceae,g_Megasphaera
9.631108	p_Firmicutes,c_Clostridia,o_Clostridiales,f_Peptoniphilaceae,g_Parvimonas
9.249647	p_Firmicutes,c_Clostridia,o_Clostridiales,f_Peptostreptococcaceae_[XI]
10.20735	p_Firmicutes,c_Mollicutes,o_Mycoplasmatales,f_Mycoplasmataceae
9.201216	p_Firmicutes,c_Clostridia,o_Clostridiales,f_Peptoniphilaceae
10.09428	p_Firmicutes,c_Mollicutes,o_Mycoplasmatales
4.250351	p_Firmicutes,c_Erysipelotrichia,o_Erysipelotrichales
4.855536	p_Bacteroidetes,c_Flavobacteriia,o_Flavobacteriales
8.66294	p_Firmicutes,c_Mollicutes
4.940307	p_Bacteroidetes,c_Flavobacteriia
3.530813	p_Firmicutes,c_Erysipelotrichia

Figure 5

Bar plots showing the differences of relative abundance

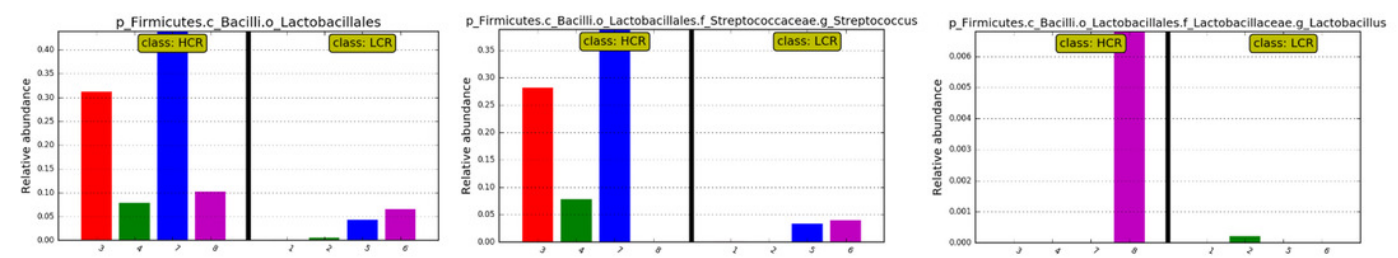



Figure 6

Cladogram showing significantly different taxa at order and class level

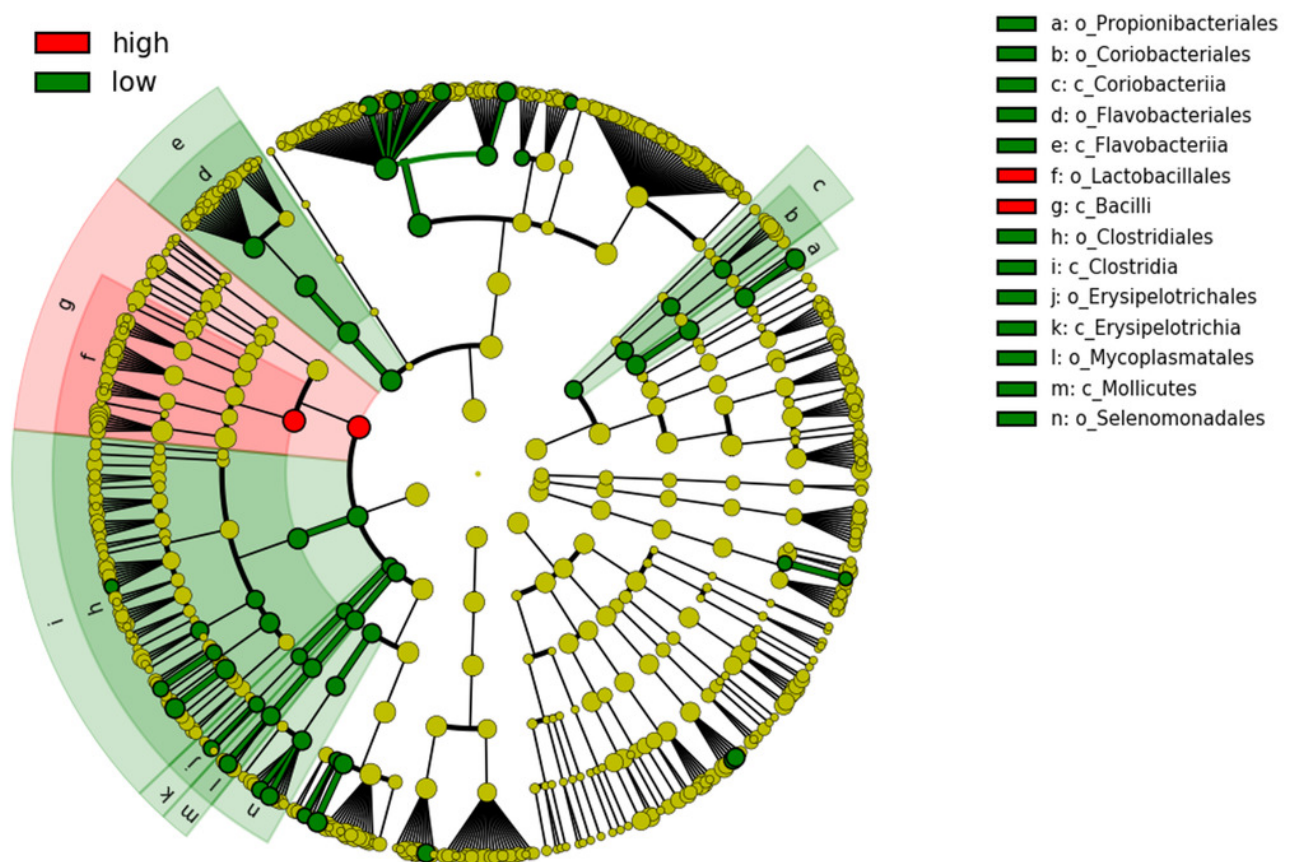
Caries risk status is delineated by the prevalence of oral bacteria. The six rings within the cladogram represent different levels within bacterial classification. Shaded circles refer to the significant taxa identified by LEfSe analysis. Red indicating bacterial groups enriched in the high caries risk group and green indicating those that are highly abundant in low caries risk group. Each circle's diameter is proportional to the taxon's abundance.

All Tables are included in this file

Table 1: shows the demographic s of study subjects characterized by caries risk

Non-malignant condition: Severe aplastic anemia, Severe congenital neutropenia, and Primary immunodeficiency disorder.

* WHO grade ●*Grade 0 (none)– No oral mucositis*
        ●*Grade 1 (mild)– Erythema and soreness*
        ●*Grade 2 (moderate)– Ulcers; able to eat solid food*
        ●*Grade 3 (severe)– Ulcers; but requires liquid diet (due to mucositis)*
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Table 2: Association with BSI

*Odds ratio

A value <1 implies that the factor is negatively associated with (i.e. protects against) the outcome. The percentage decrease in the odds (OR) is (1 - OR) × 100.
A value >1 implies that the factor is positively associated with (i.e. increases the risk of) the outcome. The percentage increase in the odds (OR) is (OR - 1) × 100.
**95%-confidence interval.

Factor	Estimate	OR*	95%CI**	p-value
(Intercept)	-0.96	0.38	0.05 - 2.09	0.30
Age	-0.13	0.88	0.72 – 1.02	0.12
Caries risk	3.06	21.39	2.44 – 427.55	0.02*
Mucositis grade	-0.49	0.61	0.25 – 1.32	0.22

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50 **LEfSe)**

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