e-ISSN: 3027-0650
Vol. 3, Issue 1, 578-589., October 30-Novermber 1, 2025

COMPARATIVE METAGENOMIC ANALYSIS OF THE ORAL MICROBIOME IN DIABETIC AND NON-DIABETIC INDIVIDUALS

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Abstract
Diabetes has been associated with alterations in the oral microbiome, but the specific relationship remains unclear. This study compared the oral microbiomes of 34 diabetic and non-diabetic individuals using oral swab samples. DNA was extracted, quantified, and sequenced with PacBio Sequel IIe technology targeting the 16S rRNA V1–V9 regions. Data were analyzed with QIIME2, and statistical tests assessed diversity and group differences. Results showed that pathogenic genera such as Pseudomonas and Klebsiella were more abundant in diabetics (31.2% vs 18.7% for Pseudomonas). Alpha diversity was lower in diabetic samples (Shannon index 1.58 vs 2.20), though no significant difference in total bacterial abundance was found (p=0.998). Overall, the findings suggest diabetes is linked to shifts in oral microbiome composition, and further research with larger cohorts is needed to confirm these patterns.

Keywords: Type 2 Diabetes; Oral Microbiome; Periodontal Bacteria; Insulin Resistance; Metagenomics.

1 Introduction

With over half a billion people affected globally, diabetes mellitus (DM) is one of the world's fastest-growing diseases [1]. It is a group of metabolic disorders identified by elevated blood glucose levels, which is a condition known as hyperglycemia. Insulin, a peptide hormone that is released by the pancreatic β cells, maintains normal blood sugar levels by facilitating the uptake of glucose into our cells. Its release is triggered by elevated blood sugar levels in the blood. Insulin impairment varies significantly depending on the type of diabetes. According to the American Diabetes Association (ADA), DM is primarily classified into four types: type 1, type 2, gestational, and diabetes associated with other causes [2].

The oral cavity is home to a diverse number of microorganisms including bacteria, fungi, viruses, and archaea. Its vast microbial community is surpassed only by that of the gut. Several studies have shown a difference in the oral microbial composition of diabetic and non-diabetic individuals. In a Chinese study examining the oral microbiome of type 2 diabetics and healthy controls, it was discovered that the patients had lower levels of Acinetobacteria and higher levels of *Neisseria*, *Streptococcus*, *Haemophilus*, and *Pseudomonas* genera [3]. Additionally, diabetes is associated with oral health complications such as caries and gum disease. Diabetic patients were found to have more active caries with higher levels of *Streptococci* and *Lactobacilli* in their supragingival plaque [4]. In diabetic patients with no oral diseases, there were higher abundances of the periodontal pathogens *Porphyromonas gingivalis* and *Prevotella melaninogenica* suggesting their role as biomarkers for oral diseases in T2DM [5].

Gum diseases such as gingivitis and periodontitis are particularly common in diabetics, with an estimated 80% prevalence [6]. This is usually attributed to increased salivary glucose which leads to the overgrowth of certain pathogenic bacteria. Longo et al. investigated how glycaemic status in T2DM patients affected subgingival microbiota associated with

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periodontitis [7]. They divided 21 T2DM patients with chronic periodontitis based on glycemic control into adequate (Hba1c <7.8%) and inadequate (Hba1c ≥8%) groups, and found that inadequate glycaemic control favored fermenting oral bacterial species and increased potentially invasive pathogens which altered the subgingival microbiome in the patients with poor glycemic control.

Another study assessed the bacterial composition in plaque samples from South Africans with periodontal disease across various glycaemic statuses using 16S rDNA sequencing [8]. Fusobacteria and Actinobacteria were found to be more abundant in people with diabetes, while Proteobacteria were less abundant. Using conditional regression models the study found that Actinobacteria increased the odds of diabetes by 10% in subjects' patients with gingival bleeding while Fusobacteria increased these odds by 14%. Conversely, another study that used 16S rRNA sequencing did not discover any significant differences between the oral microbiomes of diabetes patients and controls [9]. The only exceptions were the class Synergistia and the genus TG5 associated with periodontitis, which were more common in the control group, implying that the relationship between the oral microbiome and T2DM may be driven by lifestyle and nutrition rather than diabetes itself. In Nigeria, while metagenomic analysis of oral microbiome in healthy individuals have been reported [10], to our knowledge, there is dearth of report on the metagenomic analysis of oral microbiome in diabetic individuals. Hence, the aim of this study is to analyze the oral microbiome of diabetic and non-diabetic individuals to determine the relative and differential abundance of specific bacteria.

2 Materials and Methods

2.1 Ethical Considerations

The study was approved by the American University of Nigeria and Federal Medical Center, Yola's Institutional Review Board (IRB) following the National Code of Health Research Ethics. All participants were asked for their informed consent.

2.2 Sample Collection

A total of 34 oral samples were collected for this study. The samples were collected using a sterile oral swab. Participants were instructed to thoroughly swipe the swab across all the surfaces of their teeth. The study excluded participants who were under the age of 18 or are pregnant.

2.3 DNA Extraction and Pooling

DNA was isolated from oral samples using the Zymo Research Quick Miniprep DNA Extraction Kit. After collection, the swab was rinsed in 500 µl of Genomic Lysis Buffer while vortexing for 4-6 seconds, followed by a brief incubation at room temperature for 5-10 minutes. The mixture was then transferred to a Zymo-Spin™ IICR Column and centrifuged to separate cellular debris from the DNA. Subsequent wash steps were performed using DNA Pre-Wash Buffer and g-DNA Wash Buffer to purify the DNA. Finally, the DNA was eluted using DNA Elution Buffer and then stored at -20°C.

Following DNA extraction and quantification using the Nanodrop spectrophotometer, six individual control samples were pooled together to create one pooled control sample. The remaining 28 samples were pooled into separate pools of approximately equal amounts to generate a total of 9 pooled samples. All pooled samples were based on participant age categories.

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2.4 16S rRNA Sequencing and Analysis

Genomic DNA was sent to Inqaba Biotechnical Industries for 16S rRNA gene sequencing. Bacterial DNA samples were PCR amplified using universal primers 27F and 1492R targeting the V1-V9 region. Amplicons were barcoded with PacBio M13 barcodes using limited-cycle PCR for multiplexing. The barcoded amplicons were quantified and pooled in equimolar amounts. AMPure XP bead-based purification was performed. A PacBio SMRTbell library was prepared from the pooled amplicons according to the provided manufacturer's protocol. Primer annealing and polymerase binding were performed according to the SMRTLink software protocol to prepare the library for sequencing on the PacBio Sequel IIe system. The relative abundance of identified bacterial taxa was determined using QIIME2 [11].

2.5 Statistical Analysis

Relative abundance data, expressed as percentages, was used to calculate alpha diversity indices, specifically the Shannon index in using Excel. Log_2 fold change (Log_2FC) was used to quantify differences in community composition between groups. Beta diversity was assessed using Bray – Curtis Dissimilarity. A t-test was performed to evaluate the statistical significance of differences in overall relative abundance between the control and groups using SPSS. A significance level of $p < 0.05$ was used to determine statistical significance.

3 Results

3.1 Relative Abundance of Identified Bacteria

Non-diabetic individuals exhibited relatively consistent levels of the dominant genera (*Pseudomonas*, *Ochrobactrum*, and *Klebsiella*) identified, whereas diabetic individuals showed greater variability in their relative abundances.

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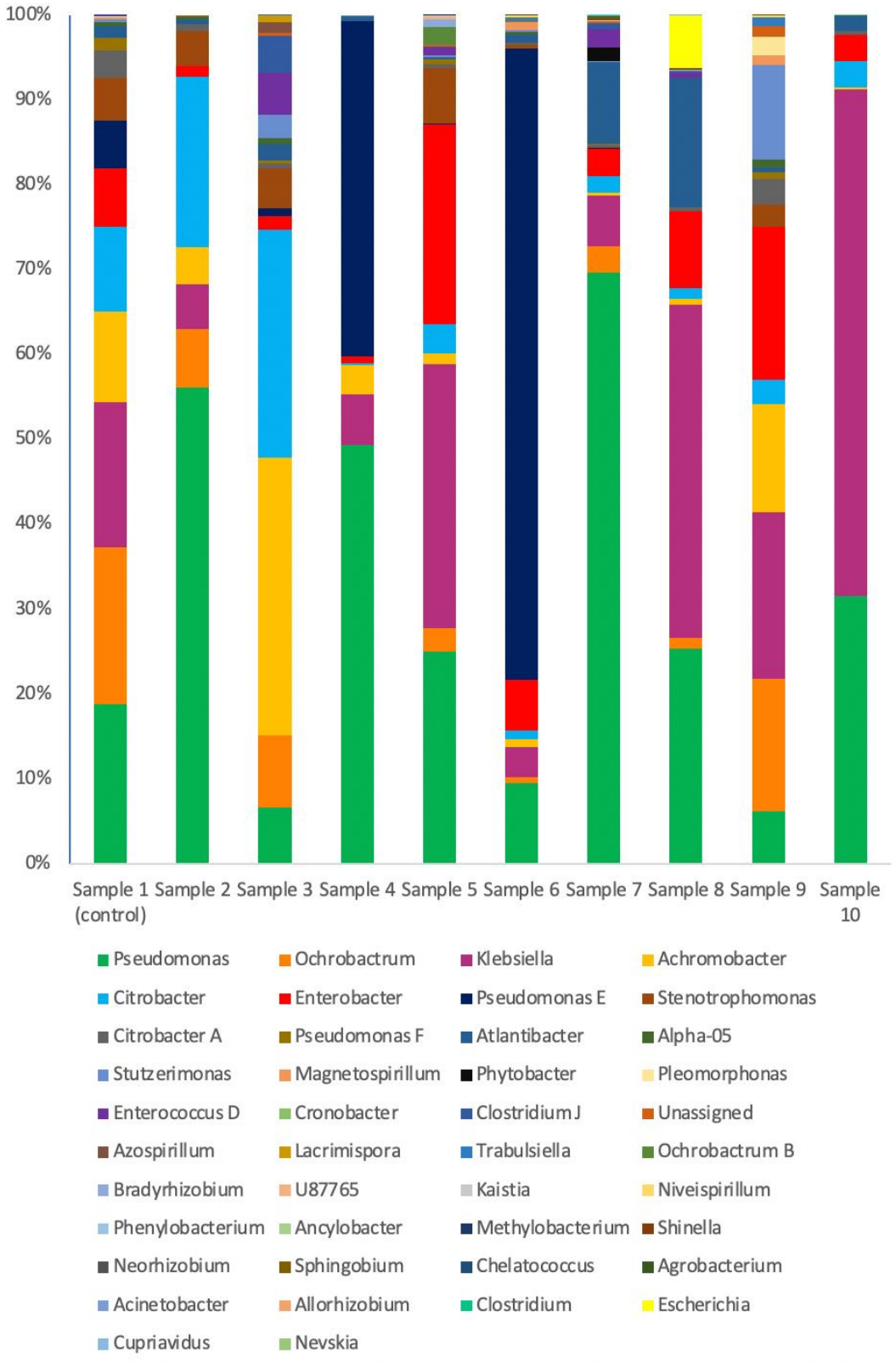

Figure 1 Relative Abundance of Identified Bacteria Taxa Across Samples

Overall, diabetic samples exhibited higher relative abundances of *Pseudomonas* (31.16% vs. 18.74%) and *Klebsiella* (18.80% vs. 17.07%) compared to the control. *Pseudomonas E* was also enriched in diabetic samples (12.65% vs. 5.66%), while *Ochrobactrum* was markedly reduced (4.36% vs. 18.53%). Other genera, including *Citrobacter* and *Achromobacter*, showed modest differences between groups, whereas

Stenotrophomonas, *Citrobacter A*, and *Pseudomonas F* were relatively rare in the diabetic samples compared to the control (Figure 1, Table 1).

Table 1 Top 10 Most Abundant Bacterial Genera in Test Samples Compared to Control (as %)

Taxon	**Control (%)**	**Avg. of Test Samples (%)**
Pseudomonas	18.74%	31.16%
Klebsiella	17.07%	18.80%
Pseudomonas E	5.66%	12.65%
Citrobacter	9.95%	6.68%
Enterobacter	6.91%	7.84%
Ochrobactrum	18.53%	4.36%
Achromobacter	10.68%	6.32%
Stenotrophomonas	5.02%	2.03%
Citrobacter A	3.16%	1.14%
Pseudomonas F	1.51%	0.19%

3.2 Differential Abundance of Identified Bacteria

Diabetic samples had higher abundances of *Enterococcus D* ($Log_2FC = 6.765$) and *Clostridium J* (9.279), and lower abundances of *Ochrobactrum* (-2.219), *Citrobacter A* (-3.779), and *Pseudomonas F* (-3.611) (Table 2).

Table 2 Differentially Abundant Bacterial Taxa Based on Log_2 Fold Change (Log_2FC)

Taxon	Log2FC
Ochrobactrum	-2.219
Pseudomonas E	1.045
Stenotrophomonas	-1.466
Citrobacter A	-3.779
Pseudomonas F	-3.611
Atlantibacter	3.394
Alpha-05	-1.274
Stutzerimonas	3.72
Phytobacter	1.073
Pleomorphonas	2.022
Enterococcus D	6.765
Cronobacter	-9.274
Clostridium J	9.279

3.3 Alpha Diversity between Samples

Shannon diversity (H) across the ten samples ranged from 1.002 to 2.266, with Sample 1 (control) exhibiting the highest diversity (2.205) and Sample 10 the lowest (1.002). The mean H was 1.58 ± 0.15 (SE), the median was 1.52, and the interquartile range was 0.98 (Figure 2, Table 3).

Table 3 Shannon Diversity Index (H) of the Different Sample Types

Sample Type	Shannon Index (H)
Sample 1 (control)	2.205
Sample 2	1.410
Sample 3	2.020

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Sample 4	1.095
Sample 5	1.806
Sample 6	1.070
Sample 7	1.252
Sample 8	1.639
Sample 9	2.266
Sample 10	1.002

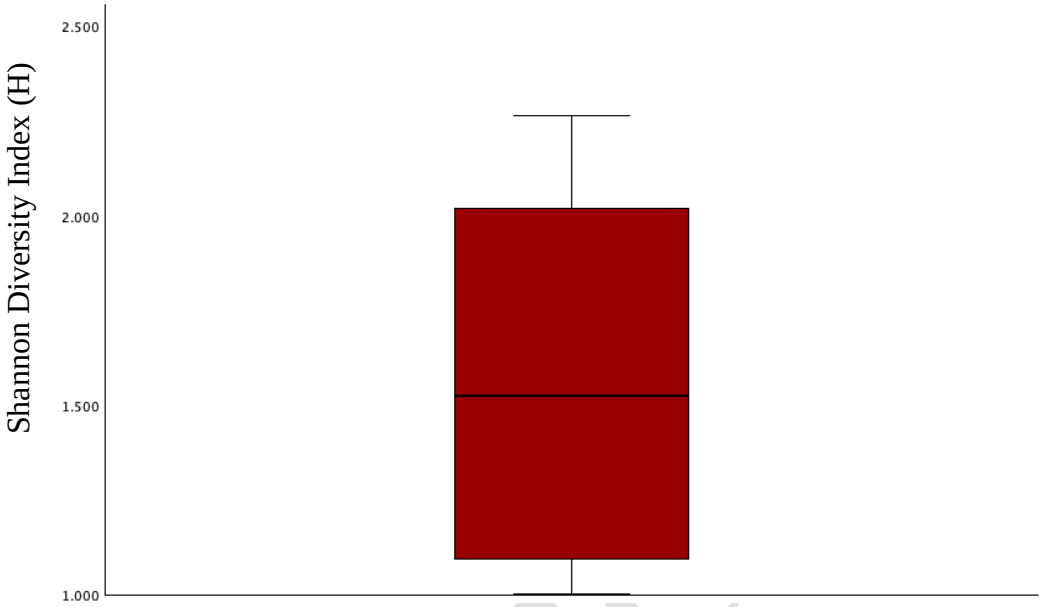

Figure 2(A) Boxplot of the Shannon Diversity Index of the Samples

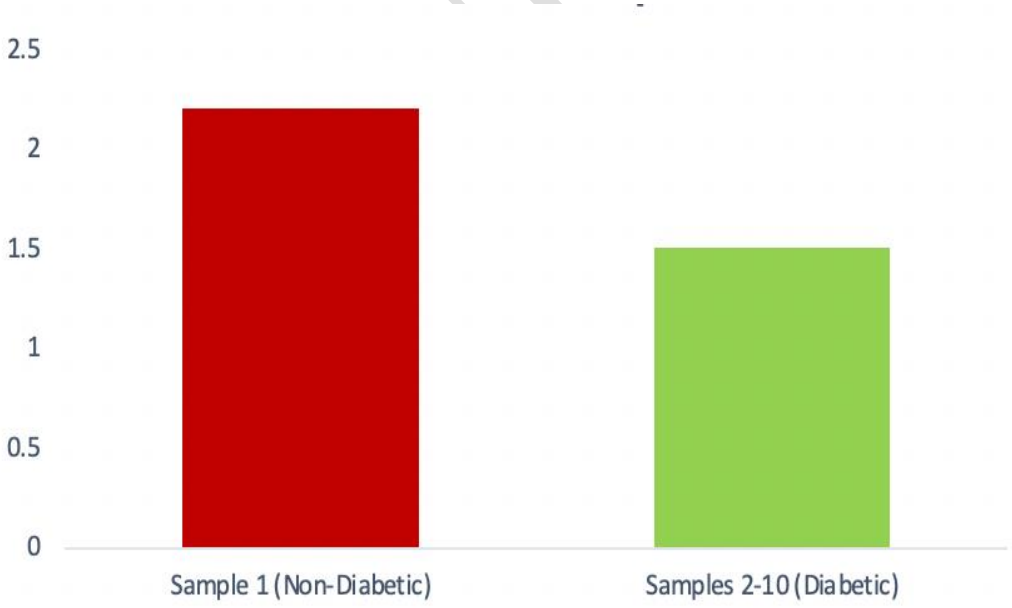

Figure 2(B) Shannon Diversity Index Comparison Between the Control and the Other Samples (avg.)

3.4 Beta Diversity between Samples

The Bray-Curtis dissimilarities between Sample 1 (control) and the other samples ranged from 0.325 to 0.702, with the lowest dissimilarity observed between Sample 1 and Sample 9 and the highest between Sample 1 and Sample 6 (Figure 3, Table 4).

Table 4 Bray – Curtis Dissimilarity between Sample 1 (Control) and Other Samples

Sample Comparison	Bray-Curtis Dissimilarity
Control vs. Sample 2	0.476114251
Control vs. Sample 3	0.539049108
Control vs. Sample 4	0.643781483
Control vs. Sample 5	0.431569891
Control vs. Sample 6	0.702008884
Control vs. Sample 7	0.644591843
Control vs. Sample 8	0.521129297
Control vs. Sample 9	0.325279749
Control vs. Sample 10	0.559724077

The mean dissimilarity score was 0.538 (95% CI: 0.448–0.629), with a median of 0.539. The standard deviation was 0.118, and the interquartile range was 0.191. The minimum and maximum dissimilarity scores were 0.325 and 0.702, respectively.

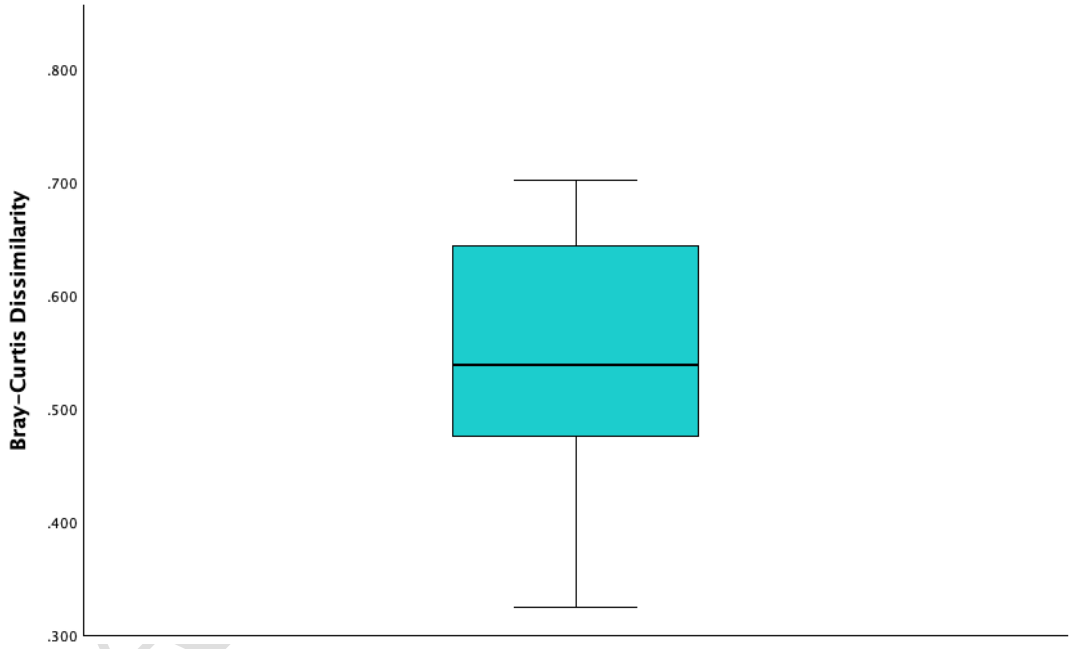

Figure 3 Bray-Curtis Dissimilarity Boxplot

4 Discussion

The relationship between diabetes and the oral microbiome has garnered considerable attention, with evidence suggesting that diabetes can influence microbial composition in various parts of the body, including the oral cavity [12]. The data revealed notable shifts in the abundance of several bacterial genera between the diabetic and non-diabetic groups, although these findings are based on pooled samples (10 samples from 34 participants). For example, *Pseudomonas* was more prevalent in the diabetic group (31.16%) compared to the non-diabetic group (18.74%), as seen in Table 1. Similarly, in a study in China, patients with type 2 diabetes exhibited higher levels of *Pseudomonas*, alongside *Neisseria*, *Streptococcus*, and *Haemophilus* [3]. The data analyzed from this study showed also that *Klebsiella* and *Pseudomonas E* were

more abundant in the diabetic group, while genera like *Ochrobactrum*, *Stenotrophomonas*, and *Pseudomonas F* showed lower relative abundances in the same group (Figure 1).

Both *Pseudomonas* and *Klebsiella* are associated with pathogenicity and chronic infections, which aligns with previous studies that have identified these bacteria in individuals with compromised health [13]. *Pseudomonas* in particular is a well-known opportunistic pathogen, often implicated in infections in individuals with weakened immune systems. For example, a study of cystic fibrosis patients found that chronic *P. aeruginosa* colonization was the main cause of severe lung damage [14]. The same study detected identical bacterial strains in both oral (saliva and gum plaque) and lung (sputum) samples, proving the mouth can serve as an infection reservoir for vulnerable hosts. Additionally, patients with P. aeruginosa infections showed reduced microbial diversity, showing its ability to dominate in immunocompromised individuals. *Klebsiella*, has also been shown migrate from the mouth to the gut where it could cause inflammation, contributing to irritable bowel disease [15,16]. The observed increase in *Pseudomonas* and *Klebsiella* suggests that diabetes may create an environment conducive to the growth of such opportunistic pathogens.

Further analysis of the differential abundance of the genera identified, measured by Log_2 Fold Change (Log_2FC), revealed that genera such as *Clostridium* and *Enterococcus* demonstrated the highest positive Log_2FC values (+9.279 and +6.765, respectively), indicating an increase in diabetic microbiomes (Table 2). *Enterococcus* has been linked to dysbiosis in other body sites, particularly the gut, where it is associated with inflammation and insulin resistance [17]. In contrast, *Clostridium* has emerged as a promising probiotic with the potential to improve blood glucose levels and gut health. In a study, *Clostridium butyricum* was shown to effectively balance blood glucose levels in diabetic male rats, reducing their levels from 206.6 ± 67.7 mg/dL to 85.1 ± 3.8 mg/dL after supplementation [18]. Additionally, another study found that administering a specific strain of *Clostridium butyricum*, known as CGMCC0313.1, to diabetic mouse models improved fasting glucose and insulin sensitivity while also decreasing blood lipids and inflammation [19].

Alpha diversity, as measured by the Shannon index (H), was consistently lower in diabetic samples (Table 3). The mean H for diabetic samples was 1.58 ± 0.15, compared to 2.205 in the non-diabetic control, as shown in Figures 2(A) and 2(B). Reduced microbial diversity is a sign of dysbiosis and has been correlated with increased disease susceptibility [20]. The least diverse sample (Sample 10, H = 1.002) may represent an extreme case of microbial depletion, possibly linked to poor glycemic control or secondary complications. A less diverse microbiome is generally less resilient as it leads to the loss of beneficial bacteria [21]. This can potentially create a feedback loop that worsens both oral and systemic health in diabetic patients.

Bray-Curtis dissimilarity analysis also revealed differences in the oral microbiome composition between diabetic and non-diabetic individuals (Table 4). The dissimilarity scores ranged from 0.325 to 0.702, with an average score of 0.538 (Figure 3). This suggests that diabetes alters the oral microbiome, but the degree of the change may vary between individuals, possibly due to how long they've had diabetes, the medications they use, or their oral hygiene habits as mentioned earlier.

Although notable differences in the composition and diversity of the oral microbiome were observed between diabetic and non-diabetic samples, statistical analysis using a two-tailed t-test revealed no significant difference in the overall relative abundance between the

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two groups (p = 0.998). This suggests that while the total amount of bacteria may be similar, the specific genera present and their distributions vary, suggesting a shift in the structure of the microbial community rather than its overall quantity.

5 Conclusion

This study highlights that diabetes is associated with notable shifts in the oral microbiome, including an increase in potentially pathogenic genera such as *Pseudomonas* and *Klebsiella*. These changes suggest a dysbiotic shift that may contribute to increased susceptibility to oral infections and systemic health complications. Additionally, the analysis revealed reduced alpha diversity in diabetic samples, evidenced by a lower Shannon index, which signals a dysbiotic shift in the microbiome. The Bray-Curtis dissimilarity analysis further confirmed alterations in microbiome composition between diabetic and non-diabetic individuals, suggesting that diabetes can modify microbial communities in the oral cavity.

Despite observing differences in microbiome composition and diversity, statistical analysis showed no significant difference in overall bacterial abundance (p = 0.998). The small sample size and use of 16S rRNA gene sequencing limit the conclusions. Future studies with larger groups and more advanced techniques are necessary to better understand the relationship between diabetes and the oral microbiome, which could help improve oral and overall health in people with diabetes.

Author Contributions

Habiba Amani Abdullahi: Methodology – investigation, data acquisition and interpretation; Writing - original draft preparation, Malachy Ifeanyi Okeke: conceptualization, supervision and project management; Methodology – Data Interpretation, Writing – review and editing.

Funding: The research received no external funding.

Conflict of Interest: The authors declare no conflict of interests.

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